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IN WHAT EMBRYONIC STAGE DO THE EGGS OF *NEODIPRION* ENTER THE WINTER DIAPAUSE?[1]

BY WOLODYMYR BRYGIDER[2]

Abstract

This paper deals with the early embryonic development of *Neodiprion banksianae* Roh., *N. nanulus* Schedl., and *N. sertifer* Geoff., up to the initiation of winter diapause. Methods of preparation are described and the course of development is traced and described with the aid of two text-figures and two plates. The three species were found to enter diapause at approximately the same stage, but some differences in the details of their embryonic structure were discovered.

Introduction

In November, 1948, Prof. C. E. Atwood of the Department of Zoology, University of Toronto, who had planned a program of research on the life histories of the Diprionidae of eastern Canada, suggested to the writer that he attempt to solve the problem indicated in the above title.

The species *Neodiprion banksianae*, *N. nanulus*, and *N. sertifer* belong to the group whose members are known to pass the winter in the egg (Atwood and Peck (1) and personal communication from C. E. Atwood). All members of the genus lay their eggs in pockets made in the needles of conifers and there is a very close association between the needle and the egg, so that the former must remain alive if the egg is to complete its development.

As material for this investigation Professor Atwood provided freshly cut branches of three species of pine, each bearing needles containing eggs of a different species of *Neodiprion*. These were as shown in Table I.

TABLE I

Species of *Neodiprion*	Host tree (*Pinus*)	Date received
N. banksianae Roh.	Jack pine *P. banksiana* Lamb.	Nov. 16, 1948
N. nanulus Schedl.	Red pine *P. resinosa* Ait.	Nov. 16, 1948
N. sertifer Geoff.	Scots pine *P. silvestris* L.	Dec. 21, 1948

[1] *Manuscript received August 28, 1951.*

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Technique

In order to avoid exposure of the eggs to the warmth of the laboratory and to prevent stimulating them to develop further, they were immediately fixed without removing them from their needle pockets. Some of the eggs were fixed in Bouin's fluid, according to the original formula, i.e., saturated picric acid, formaldehyde, and acetic acid, 15: 5: 1 (7, p. 82), by volume, and mixed immediately before using. The remaining eggs were fixed in Gilson's fluid, according to the formulae of Becker and Roudabush (2).

Both fixatives were used mostly at room temperature but were also tested at 60° C. This temperature, however, proved unsatisfactory, for, though facilitating the penetration of the fluid, it caused too great shrinkage of the egg and a wrinkling of the surface. Even at room temperature some deformation of the egg was unavoidable, but it was comparatively little.

The material was left in the fixative overnight, i.e., 16–20 hr., in order to allow for complete penetration of the liquids not only through the egg membrane but also first through the compact tissue of the needle. From Bouin's fluid the fixed eggs were transferred to 70% alcohol, which was renewed until no further change of color was visible. From Gilson's fluid the preparations were likewise transferred to 70% alcohol, but sufficient iodine in potassium iodide (7, pp. 87,88), was added, drop by drop, to give the color of cognac. After removing possible sediments of corrosive sublimate, the preparations were transferred to pure 70% alcohol, which was renewed until it became colorless. In order to remove iodine from the microscopic slides Heidenhain's method was employed, viz., the use of a 0.25% solution of sodium thiosulphate (7, p. 88).

All the fixed material was preserved in 70% alcohol until it was embedded in paraffin. Two methods of embedding were used with good results: (*a*) the methyl benzoate – celloidin method of Péterfi, and (*b*) the Dioxan method of Graupner-Weissberger.

According to the first method, the material, after being passed gradually through the higher grades to absolute alcohol, is put in a 1% solution of parlodion (celloidin) in methyl benzoate (7, pp. 107–109). For this solution I use three hermetically sealed glass jars, supported in a wooden stand. This set is kept in a large glass dish covered with a glass plate. Transferred from absolute alcohol into the first jar the pieces first float upon the surface of the solution, but after two or three hours settle to the bottom. Then they are moved into the second jar and finally into the third.

From the last jar they are transferred to benzol N-I, next into benzol N-2, and then into a saturated solution of paraffin in benzol, which is kept on the oven. Finally they are put into pure paraffin, changed three times and then embedded.

The dioxan method shortens the process, since it eliminates all the higher alcohols (7, pp. 93–94). The object is transferred immediately from 70% alcohol to dioxan, which is changed three or four times. Water and alcohol flowing down from the object in a little glass basket are then absorbed by

calcium chloride, placed on the bottom of the jar. Such a basket also facilitates the transfer of the object from one dioxan to another.

From the last dioxan the object goes into a mixture of dioxan and paraffin in the proportion of 1 : 1, then 1 : 2, which stays within the oven. Lastly it is put in pure paraffin, changed three times, and then embedded.

Paraffin blocks are difficult to cut, because of the well known fragility of egg yolk and the hardness of the leaf tissue, which surrounds the eggs. This is the reason why it is not easy to get a faultless series of sections. The best sections are those which are cut across the pine needle, next are longitudinal sections, vertical to the flat surface of the needle, worst the longitudinal sections, parallel to this surface.

The stains, prepared mostly according to Romeis and partly after Lee (6), appeared quite good.

Of the nuclear stains Mayer's acid haemalum was satisfactory, reliable, and not too fast. Ehrlich's acid haematoxylin stained much more slowly but more selectively than haemalum. Both these stains gave a very good contrast with eosin, acid fuchsin, and orange G. Of these three plasma stains acid fuchsin was the most rapid, eosin was slower but more selective, while orange G was the slowest of the three, requiring 18 to 24 hr.

By contrast is meant the difference between the yolk nuclei and yolk spherules in their affinity for the stains. Mayer's carmalum with light green gave the best contrast between these elements. Carmalum stains quickly and must therefore be controlled instantly during the staining process. Light green stains slowly and when it acts too long, it reduces the effect of the nuclear stain. Its action must, therefore, also be interrupted at a suitable moment.

The stained preparations being protected from direct sunlight, no loss of color of light green was observed, even after a year, although this stain, as is well known, does not keep at all well.

As for Heidenhain's iron haematoxylin, it shows very clearly the small nuclei of the minute and crowded cellules of the embryo. But, unfortunately, it stains the nuclei black, as well as the yolk spherules, and thus destroys all the contrast between these two elements.

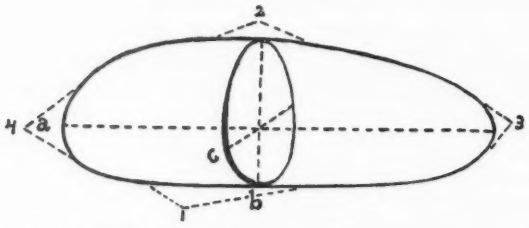
As to the rate of action of all these stains no precise statement can be given, because it depends upon several factors, viz., the fixative used, freshness of stain, its ripeness, degree of solution immediately before using, etc. The preparations were therefore always controlled with the microscope during the process of staining, in order to obtain the best results.

Description of Egg and Embryo

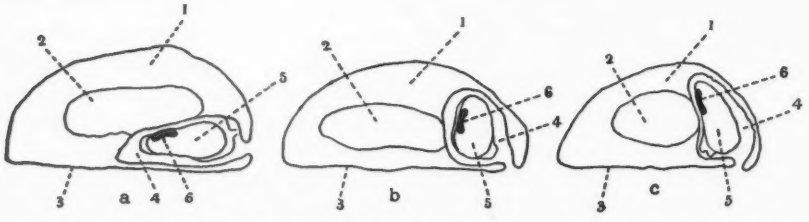
Egg

Although the egg, at the time when it enters the winter diapause, is long past the one-celled stage, it nevertheless maintains its original external shape. It is an oval spheroid, flattened on the dorsal side, convex on the ventral side, a

little larger behind and smaller in front (Text-fig. 1). A cross section shows that this spheroid is nearly twice as wide on the dorsal as on the ventral side (Text-fig. 2:5).



TEXT-FIG. 1. Egg of *Neodiprion* (Schematic). *1.* Dorsum; *2.* venter; *3.* front; *4.* rear; *a*, length; *b*, thickness; *c*, width.



TEXT-FIG. 2. Position of egg and egg pocket in pine needle. Cross section (oc. 10, obj. 1 Leitz. × 32). (Figures reduced in reproduction to three-fourths of the size of the author's originals.)

a. N. banksianae; b. N. sertifer; c. N. nanulus.

1. Tissue of pine needle; *2.* vascular bundle; *3.* lower (flat) side of needle; *4.* pocket channeled by the ovipositor; *5.* egg; *6.* embryo.

Eggs of the three species of *Neodiprion* used in this study were measured from microscopic preparations, the mean of each of the three dimensions being determined from several measurements of each (43 in all), as given in the Table II.

TABLE II

MEAN DIMENSIONS OF EGG IN MM. (NUMBER OF MEASUREMENTS IN PARENTHESES)

Dimensions	*N. banksianae*	*N. sertifer*	*N. nanulus*
Length	1.53 (5)	1.62 (3)	1.72 (4)
Thickness	0.46 (5)	0.38 (3)	0.43 (5)
Width	0.22 (4)	0.28 (5)	0.23 (9)

We would emphasize the fact that the dimensions given here have only an approximate value, since measurements of this kind should be made on fresh material and in large numbers. Such measurements are outside the aim and

scope of the present report. They are included only as an aid in the description of the egg and are significant also with regard to their number on a single pine needle and the distance between them. On this matter we may refer to Atwood and Peck (1), although *Neodiprion sertifer* is not discussed by these authors.

Each egg lies in a pocket of corresponding size and shape and channeled by the ovipositor of the female sawfly (Text-fig. 2:4). The position of this pocket and of the egg in the needle are quite different in *N. banksianae* from those of *N. nanulus* and *sertifer*, as shown in Text-fig. 2:*a*, *b*, and *c*. This position must be taken into account when we aim to cut the egg transversally, sagittally, or coronally. The cross sections of the pine needle, as shown in Text-fig. 2, are also cross sections of the egg in all three of the species. It is otherwise with longitudinal sections. Those which run parallel to the lower, flat surface of the needle cut the egg of *N. banksianae* sagittally (Text-fig. 2:*a*), but those of *N. sertifer* and *nanulus* coronally (Text-fig. 2:*b*, *c*). Also, the sections which run perpendicular to the lower, flat surface of the needle cut the eggs of *N. sertifer* and *nanulus* sagittally (Text-fig. 2:*b*, *c*) but the eggs of *N. banksianae* coronally (Text-fig. 2:*a*).

Chorion

Outwardly the egg is protected by the chorion. It consists of two structureless membranes, an external, brighter one, the exochorion, and an internal, darker one, the endochorion. In our preparations the endochorion is mostly in contact with the egg surface (Figs. 1, 4, 8:*6*; Fig. 5:*2*; Fig. 7:*4*; Fig. 10:*7*), while the exochorion is usually detached from it and from the endochorion and keeps close to the inner surface of the pocket in which the egg lies (Figs. 1, 4, 8: *5*; Fig. 5: *1*; Fig. 7: *3*). This fact may be explained as follows. After oviposition the exochorion, the egg's external membrane, is wet and may be sticky. It sticks to the inner surface of the pocket and fixes the egg in its position.

The different behavior of the two membranes to the stains differentiates them well. The exochorion is definitely acidophilic, while the endochorion shows mostly a basophilic nature, although this characteristic is not as distinct as in the first membrane. For a better orientation of the facts described above, Table III, is given.

Immediately under the chorion, in *N. banksianae* and *N. nanulus*, lies the serosa (Figs 1, 8: *7*; Fig. 10: *6*), but in *N. sertifer* a sheet of very much vacuolized plasm separates the serosa from the chorion (Fig. 4: *7*, *9*; Fig. 5: *5*, *3*; Fig. 7: *5*, *6*). Many meshes of this plasm are filled with dark brown, nearly black, droplets, which give it a very characteristic appearance (Fig. 4: *9*; Fig. 5: *3*, *4*). The same vacuolized plasm is to be found also within the egg, partly between the yolk cells (Fig. 6: *3*) and partly in the lateral bands (Fig. 4: *10*), which follow the serosa, approach the same embryo, and even penetrate the amniotic cavity (Fig. 4: *11*).

TABLE III

Stains	Exochorion	Endochorion
Mayer's acid haemalum and picrofuchsin	Yellow	Pink (rosy)
Mayer's acid haemalum and acid fuchsin	Vivid red	Bluish red
Mayer's acid haemalum and eosin	Shining red	Bluish
Mayer's acid haemalum and orange G	Yellow	Blue
Ehrlich's haematoxylin and eosin	Shining red	Bluish
Heidenhain's iron haematoxylin	Black	Pale yellow
Heidenhain's iron haematoxylin and eosin	Black	Red
Carmalum and light green	Pale green	Red

Within the egg are to be found, here and there, also large acidophilic vacuoles, which show in their middle dense agglomerations of the above mentioned dark droplets (Fig. 6: *1*, *2*).

ABBREVIATIONS: L.—Leitz, obj.—objective, oc.—ocular, prc.—periplanatic eyepiece, R.—Reichert, S.—Spencer. All the figures on the plate were drawn with a Spencer camera lucida. (Figures reduced in reproduction to three-fourths of the size of the author's orginals.)

Neodiprion banksianae.

FIG. 1. Cross section of pine needle with egg and embryo. Carmalum and light green (oc.L., prc.10, obj. 16 mm. S. ×100). *1.* Tissue of the pine needle; *2.* vascular bundle; *3.* lower, flat side of the needle; *4.* egg pocket; *5.* exochorion; *6.* endochorion; *7.* serosa; *8.* contraction of serosa (sec. dorsal organ); *9.* periplasm; *10.* yolk; *11.* embryo; *12.* amnion; *13.* amniotic cavity.

FIG. 2. Nucleus of a yolk cell in division (amitotic). Haemalum and eosin. (oc. L., prc. 10, obj. 1″/18 homog. im. 19b. R. ×1250). *1.* Chromatin network with chromatin granules; *2.* acidophilic nucleoli; *3.* acidophilic yolk spherules.

FIG. 3. A distinctly separated yolk cell. Carmalum and light green (oc. L., prc. 8, obj. 1″/18 homog. im. 19b. R. ×1000). *1.* Star-shaped nucleus with chromatin network, chromatin granules and three nucleoli; *2.* vacuolized cytoplasm; *3.* yolk spherules; *4.* empty vacuoles; *5.* vacuole filled with plasmatic network.

Neodiprion sertifer.

Fig. 4. Cross section of pine needle with egg and embryo. Ehrlich's haematoxylin and eosin (oc. L., prc. 10, obj. 16mm. S. ×100). *1.* Tissue of pine needle; *2.* vascular bundle; *3.* lower, flat side of the needle; *4.* egg pocket; *5.* exochorion; *6.* endochorion; *7.* serosa; *8.* contraction of serosa (sec. dorsal organ); *9* a special sheet of the very much vacuolized plasm between endochorion and serosa with dark brown droplets; *10.* a lateral strip of the same plasm; *11.* its penetration into the amniotic cavity; *12.* amnion; *13.* amniotic cavity; *14.* embryo; *15.* yolk.

FIG. 5. Cross section of the egg on Fig. 4 at *8*. Haemalum and orange G (oc. L., prc. 10, obj. 4mm. S. ×440). *1.* Exochorion; *2.* endochorion; *3.* special sheet of very vacuolized plasm between endochorion and serosa, with dark brown droplets; *4.* dark, brown droplets; *5.* serosa; *6.* typical flattened epithelium of serosa; *7.* cuboidal epithelium of contracted serosa (sec. dorsal organ).

PLATE I

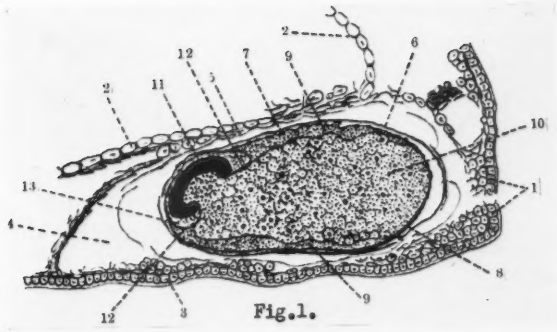


Fig.1.

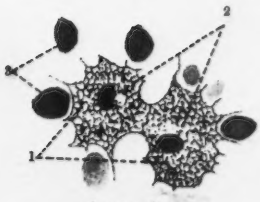


Fig.2.

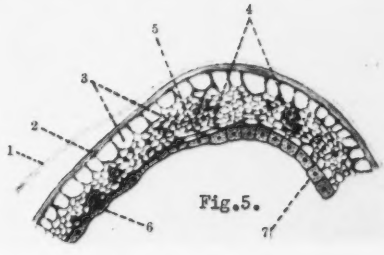


Fig.5.

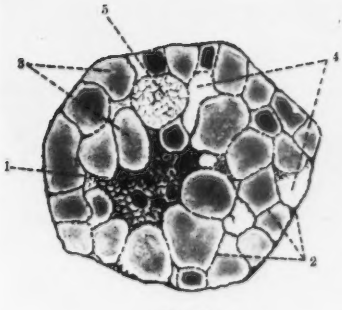


Fig.3.

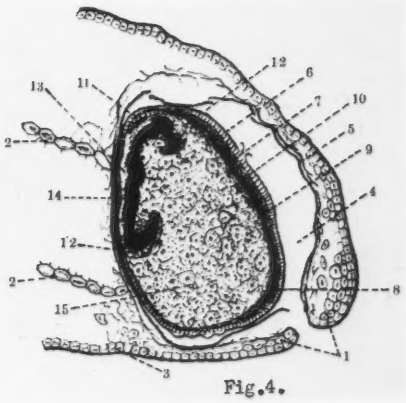


Fig.4.

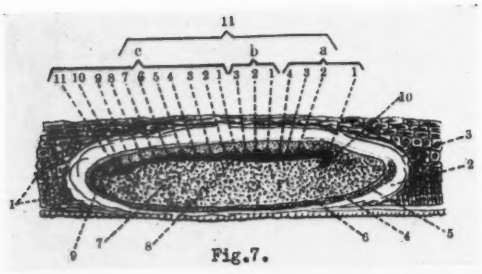


Fig.7.

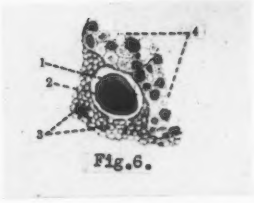


Fig.6.

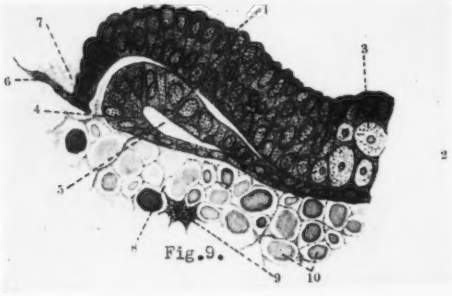


Fig.9.

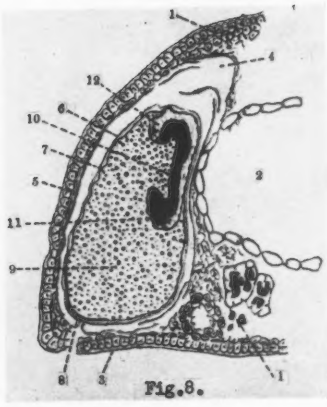


Fig.8.

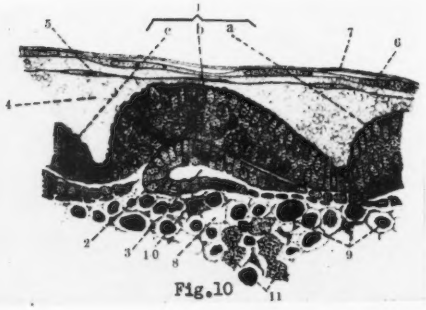


Fig.10

Serosa

The serosa has a typical appearance (Figs. 1, 4, 8: *7*; Fig. 7: *5*). It is a membrane composed of a single layer of very much flattened cells (Fig. 5: *5*, *6*; Fig. 10: *6*). Because of the minute granulated nuclei of these cells and numerous basophilic granules in their plasm, the serosa absorbs nuclear stains and always appears in the preparations in the color of these stains.

As the growing embryo absorbs the yolk, the serosa contracts. On the opposite side from the embryo its cells become shorter and thicker (Figs. 1, 4, 8: *8*). In this way the serosa takes the appearance of an almost cuboidal epithelium (secondary dorsal organ) (Fig. 5: *7*).

Periplasm

Under the serosa, i.e., between it and the yolk, we find a sheet of non-nucleated plasm, known as the *periplasm*. This layer is especially well developed laterally, as a thick layer, in *N. banksianae*. It appears here as a network with very tiny meshes (Fig. 1: *9*). In *N. sertifer* the periplasm is replaced by the same kind of vacuolized cytoplasm but with many dark brown droplets. It is likewise applied to the sides of the eggs and penetrates the amniotic cavity, where it surrounds the embryo (Fig. 4: *10*, *11*). *N. nanulus*, however,

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ABBREVIATIONS: L.—Leitz, obj.—objective, oc.—ocular, prc.— periplanatic eyepiece, R.—Reichert, S.—Spencer. All the figures on the plate were drawn with a Spencer camera lucida. (Figures reduced in reproduction to three-fourths of the size of the author's originals.)

Neodiprion sertifer.

FIG. 6. Large acidophilic vacuole with dense agglomeration of dark droplets in its middle. (Cross section of egg.) Haemalum and orange G (oc. L., prc. 10, obj. 7 L. ×775). *1.* Acidophilic vacuole; *2.* agglomeration of dark droplets; *3.* vacuolized plasma with dark droplets; *4.* two yolk cells with vacuolized cytoplasm and yolk spherules.

FIG. 7. Segmentation of embryo in sagittal section. Ehrlich's haematoxylin and acid fuchsin (oc. 10, obj. 1 L. ×32). *1.* Tissue of pine needle; *2.* egg pocket; *3.* exochorion; *4.* endochorion; *5.* serosa; *6.* sheet of vacuolized plasm between endochorion and serosa; *7.* yolk; *8.* embryo; *9.* amnion; *10.* amniotic cavity; *11.* regions of body: *a*, head, *b*, thorax, *c*, abdomen; a_1, head, a_2, mandibular, a_3, 1st maxillary, a_4, 2nd maxillary (labial) segments; b_{1-3}, pro-, meso-, and metathoracic segments (three pairs of the legs); c_{1-11}, abdominal segments (c_{11}, telson).

Neodiprion nanulus.

FIG. 8. Cross section of pine needle, egg, and embryo. Haemalum and orange G (oc. L., prc. 10, obj. 16mm. S. × 100). *1.* Tissue of pine needle; *2.* vascular bundle; *3.* lower, flat side of needle; *4.* egg pocket; *5.* exochorion; *6.* endochorion; *7.* serosa; *8.* contraction of serosa (sec. dorsal organ); *9.* yolk; *10.* embryo; *11.* amnion; *12.* amniotic cavity.

FIG. 9. Embryo in cross section. Haemalum and eosin. (oc. L., prc. 10, obj. 1″/12 oil immers. 18b R. × 1010). *1.* Ectoderm; *2.* neural groove; *3.* large nerve cells (neuroblasts) of the outer neural ridge (nerve strand); *4.* mesoderm; *5.* coelomic cavity; *6.* amnion; *7.* amniotic cavity with vacuolized periplasm; *8.* yolk; *9.* yolk nucleus; *10.* yolk spherules.

FIG. 10. Part of the embryo in sagittal section. Haemalum and eosin. (oc. 4, obj. 1″/12 oil immers., 18b. R. × 404). *1.* Ectoderm: *a*, mandibular, *b*, maxillary, *c*, labial segments; *2.* mesoderm; *3.* maxillary coelomic sac; *4.* amniotic cavity, filled with protoplasm; *5.* amnion; *6.* serosa; *7.* endochorion; *8.* yolk; *9.* yolk spherules; *10.* yolk nucleus with nucleolus; *11.* yolk nuclei in fission.

shows very little of the periplasm. The yolk is in almost direct contact with the serosa (Fig. 8). Only in the amniotic cavity does the periplasm appear more abundant (Fig. 8: *12*; Fig. 10: *4*). But here it has the structure of a net with larger meshes than in *N. banksianae* (Fig. 9: *7*). Some of these meshes are filled with acidophilic fluid.

Yolk (Fig. 1: *10*; Fig. 4: *15*; Fig. 7: *7*; Fig. 8: *9*).

The whole yolk is divided into cells, separated distinctly from one another (Fig. 6: *4*; Fig. 3). Each of them contains a very vacuolized cytoplasm, which appears in optical section as a basophilic network (Fig. 3: *2*; Fig. 6: *4*). The meshes of this net are filled with yolk spherules (Fig. 3: *3*; Fig. 6: *4*; Fig. 9: *8*, *10*; Fig. 10: *8*, *9*). They are acidophilic and stain vividly with plasma stains and also with Heidenhain's iron haematoxylin. Some of the yolk spherules are dense and dark, the others thin and pale. In many cases the meshes of this network are empty or filled with cytoplasm (Fig. 3: *4*, *5*). The presence of a great number of basophilic microgranules in the cytoplasmatic network explains why the latter appears always in the color of nuclear stains.

The nucleus of a yolk cell generally lies in the middle and is rarely round. It is usually star-shaped, produced into longer or shorter points. Within the nucleus is a chromatin network with chromatin granules and one or more acidophilic nucleoli (Fig. 2: *1*, *2*; Fig. 3: *1*; Fig. 9: *9*; Fig. 10: *10*).

This structure of the yolk cell appears best in preparations stained, as already mentioned, with carmalum and light green (Fig. 3). Here the protoplasmatic network with its microgranules and nucleus becomes red, while the yolk spherules appear in several tints of green. In this stain combination the nucleoli appear always red, never green, but in other combinations, e.g., acid haemalum with orange G, eosin, or acid fuchsin, they appear yellow and red respectively, while the nuclei with their chromatin network are blue. But in both stain combinations the nuclei show no structure; they are always like a structureless spot (Fig. 2: *2*; Fig. 3: *1*; Fig. 10: *10*).

We often find that in the microscopic preparations the yolk nuclei divide as in (Fig. 2). This seems to indicate fission, i.e., amitotic division (Fig. 10: *11*).

Embryo

The egg of *Neodiprion*, in the stage described here, contains a well developed embryo (Fig. 1: *11*; Fig. 4: *14*; Fig. 7: *8*; Fig. 8: *10*). The position of this embryo is very characteristic. It always lies laterally with one side parallel to the ventral surface of the egg. Thus the position of the embryo is never completely ventral. The side of the egg on which the embryo lies is always directed to the center of the needle, never to the outside. It may be that in this way the embryo is better protected against cold winds and sudden changes of temperature.

A knowledge of the position of the embryo, as here described, is very important, in order to obtain a particular kind of section. Only cross sections of the egg are also cross sections of the embryo in all of the three species. But the sagittal sections of the egg will be coronal for the embryo, i.e., it cuts the

embryo from the left to the right side. The coronal section of the egg however will be sagittal for the embryo, i.e., it cuts it along from dorsum to venter. Table IV, given below, shows the relations described above between the sections for (*a*) the pine needle, (*b*) the egg, and (*c*) the embryo. (Text-fig. 2: *a*, *b*, *c*).

TABLE IV

RELATIONS BETWEEN THE SECTIONS

(*a*) Pine needle	(*b*) Egg	(*c*) Embryo
I. Cross section	Cross	Cross
II. Longitudinal section: parallel to the lower (flat) side of the needle	Sagittal for *N. banksianae* Coronal for *N. sertifer* and *N. nanulus*	Coronal for *N. banksianae* Sagittal for *N. sertifer* and *N. nanulus*
III. Longitudinal section: vertical to the lower (flat) side of the needle	Coronal for *N. banksianae* Sagittal for *N. sertifer* and *N. nanulus*	Sagittal for *N. banksianae* Coronal for *N. sertifer* and *N. nanulus*

A detailed investigation of the embryo must be postponed until the opportunity is available for a study of the stages from the beginning. Here only the following observations will be made.

The embryo is surrounded by the amnion (Fig. 1, 8: *11*; Fig. 4: *12*; Fig. 7: *9*; Fig. 9: *6*; Fig. 10: *5*), a very tiny membrane, identical in structure with the serosa, of which it is a fold. The amnion is best observed in the cross section of the egg, on both sides of the embryo. About the embryo the amnion usually adheres so tightly to the serosa that it is difficult to observe it. The embryo thus lies in an amniotic cavity, which is closed on its dorsal side and at both ends, and is partly filled with a fluid, partly with a more or less vacuolized plasm (periplasm) (Figs. 1, 4: *13*; Fig. 7: *10*; Fig. 8: *12*; Fig. 9: *7*; Fig. 10: *4*). The whole yolk lies under the embryo and is in direct contact with it. The yolk cells never thrust themselves upwards between the embryo and the serosa. Thus the position of the embryo is typically *superficial*, but never immersed. Our impression is that the embryo is overgrown by the amnion and, because of this fact must be reckoned as an example of *overgrown* ("ueberwachsen") embryos, never as one of *invaginated* embryos. Nevertheless the embryo is immersed rather deeply in the yolk at its anterior and posterior ends.

It is known that *segmentation* begins very early in insect embryos. It is observed very early in the eggs of *Neodiprion* and is very distinct. In Fig. 7: *11*, which shows an embryo of *N. sertifer* in sagittal section, the segments of the head region, thorax, and abdomen are all clearly distinguishable.

As for the *embryonic layers* they are undoubtedly differentiated in this winter stage of the egg and show in the mesoderm the anlage of the coelomic cavity (Fig. 9: *1*, *2*, *3*, *4*, *5*; Fig. 10: *1*, *2*, *3*).

Summary

1. Hibernating individuals of the species *Neodiprion banksianae*, *N. nanulus* and *N. sertifer* pass the winter as well developed embryos, lying paraventrally but laterally and facing the middle of the pine needle.

2. The embryos are distinctly segmented, the embryonic layers are differentiated, and the rudiments of the coelomic cavities have appeared in the mesoderm.

3. Surrounded by the amnion, the embryos lie on the yolk, which is divided at that time into distinctly separated yolk cells. The whole is surrounded by serosa and chorion, the latter differentiated into endo-and exochorion.

4. *Neodiprion banksianae* differed from *N. nanulus* and *N. sertifer* in its positional relation to the pine needle in which the egg rests while development takes place. Minor differences of structure were also noted among the three species.

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Lastly I want to express my cordial thanks to Prof. E. M. Walker for his careful preparation of the final draft of this paper.

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* A detailed bibliography will appear with the publication of the writer's further work on the embryology of *Neodiprion*. Only the more comprehensive works consulted in *this preliminary report* are listed here.

A SIMPLE ULTRAMICROMETHOD FOR THE QUANTITATIVE ESTIMATION OF AMINO ACIDS BY PAPER PARTITION CHROMATOGRAPHY[1]

By Jacques L. Auclair[2] and Robert Dubreuil[3]

Abstract

A new ultramicromethod for the quantitative estimation of amino acids by paper partition chromatography is described. The material required and the procedure adopted are detailed. As an application of this new method, a quantitative estimation of the free amino acids present in the blood of the last larval instar of *Galleria mellonella* (L.) is presented. The results obtained, when converted into total amino nitrogen, compare favorably with total amino nitrogen results already published in the literature.

Introduction

Subsequent to the development of the method of paper partition chromatography by Consden, Gordon, and Martin in 1944 (2), several workers have published reports of techniques adapted from the above method for the quantitative analysis of amino acids. Many of these techniques involve rather elaborate operations that often require the use of costly equipment.

For the past two years, experiments were carried out on a method of quantitative amino acid analysis based on the sensitivity of the ninhydrin reaction (5). It is felt that this new method may be of practical use for several workers interested in an ultramicro quantitative estimation of amino acids encountered in biological material. The procedure is very simple and it requires no equipment other than that ordinarily utilized for paper chromatography. Acceptable accuracy of results has already been obtained with amounts of amino acid as small as 0.05 μgm. The object of this preliminary report is to describe briefly the present ultramicromethod and to demonstrate its application in the analysis of biological material, in this particular instance, insect blood.

Materials and Methods

The necessary procedure consists in the careful determination of the quantity of an amino acid that will barely give a detectable color reaction with ninhydrin on a developed two-dimensional chromatogram. Under standardized conditions, this minimum quantity of each amino acid can be determined by preparing series of two-dimensional chromatograms with gradually decreasing amounts of a mixture of pure amino acids in solution. In such series, the amount of each amino acid deposited on each paper is gradually decreased from a point above the minimum quantity to a point just below that minimum.

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Contribution from the Institut de Biologie générale et de Zoologie, Université de Montréal, Montréal 26, Qué. This investigation was supported in part by a grant from the National Research Council of Canada.

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The series are run simultaneously and after the ninhydrin reaction, the quantity just above that at which each amino acid spot ceases to be visible in standardized transmitted light is recorded. This recorded minimum quantity, different for each amino acid, serves as the basis for the quantitative estimation of each amino acid in unknown samples.

In practice, the series of two-dimensional chromatograms are developed by capillary ascent according to the method of Williams and Kirby (8). With this simple method, one can prepare rapidly series of 20 to 30 two-dimensional chromatograms ($11\frac{1}{4} \times 17$ in.) and run them simultaneously in the solvents. Phenol–water is used in the larger dimension and collidine–lutidine–water in the second dimension. The amino acids are revealed with 0.1% ninhydrin in *n*-butanol. The first solvent mixture, phenol–water, is prepared by melting crystalline phenol at 45°C. and adding distilled water, one part to four parts of liquefied phenol by volume. The best results were obtained using only freshly prepared phenol–water mixture, the grade of phenol being Mallinkrodt U.S.P. XIV. The second solvent mixture, collidine–lutidine–water, is prepared by thoroughly mixing refined 2,4,6-collidine, refined 2,4-lutidine and distilled water, 1:1:2, and collecting the upper phase after the mixture has been placed for a few hours in the oven at 35°C. The collidine and lutidine were obtained from Koppers Company, Pittsburg, Penn. Both solvent mixtures thus prepared are unsaturated with water at room temperature and assure consistent and satisfactory results. Following the phenol run, which under normal conditions lasts from 36 to 40 hr., the papers are dried overnight in a current of air at room temperature. Following the "collidine" run, which lasts usually 12 hr., the chromatograms are dried in the same way. Heating the wet chromatograms above room temperature often results in a partial destruction of the amino acids. The dried chromatograms are sprayed with the ninhydrin solution and the color reaction is allowed to go near completion by placing the papers in an oven at 70°C. for 15 min. The ninhydrin-developed chromatograms are always re-examined over transmitted light 24 and 48 hr. later. The quantitative development with ninhydrin usually requires several hours and re-examination is essential for consistent results.

For the determination of the minimum quantity of each amino acid, fresh solutions containing several amino acids are made in 10% isopropyl alcohol (1). The stock solutions are prepared at the concentrations given in Table I. Aliquots from the stock solutions are diluted 1:10 with 10% isopropyl alcohol, except for Solution III which is diluted 1:5. Further dilution of the solutions is sometimes necessary for greater accuracy of results, especially when micropipettes below 1 μl. capacity are not available. With such solutions, it is possible to prepare rapidly several series of standard chromatograms containing all the amino acids in gradually decreasing amounts.

The minimum quantity of each amino acid detected by the ninhydrin reaction on two-dimensional chromatograms is given in Table II. The data represent the average of five series of two-dimensional chromatograms and the maximum variations observed in the five series.

TABLE I

STANDARD SOLUTIONS OF AMINO ACIDS IN 10% ISOPROPYL ALCOHOL

Solution	Mgm. per ml.	Solution	Mgm. per ml.
Solution I		Solution II	
Alanine	0.2	Threonine	0.7
α-Amino-*n*-butyric acid	0.4	Leucine	0.8
Aspartic acid	0.4	Hydroxyproline	1.6
β-Alanine	0.6	Asparagine	1.0
Glutamic acid	0.2	Lysine*	1.8
Glycine	0.2	Methionine sulphoxide	1.6
Serine	0.2	Glutamine	0.5
Taurine	0.6		
Valine	0.3	Solution III	
Isoleucine	0.8	Tryptophan	1.0
Proline	1.5	Phenylalanine	1.0
		Arginine*	1.0
		Histidine*	5.0
		Solution IV**	
		Tyrosine	1.0
		Cystine***	1.0

**Hydrochlorides of these amino acids were used.*

***Solution IV is acidulated with hydrochloric acid.*

****Cysteic acid was prepared from cystine according to Dent* (3).

TABLE II

MINIMUM QUANTITIES OF AMINO ACIDS DETECTED WITH THE NINHYDRIN REACTION IN FIVE SERIES OF TWO-DIMENSIONAL CHROMATOGRAMS

Amino acid	Average minimum quantity detected, μgm.	Maximum variation in the five series	
		μgm.	%
Alanine	0.06	0.01	17
α-Amino-*n*-butyric acid	0.12	0.01	8
Arginine	4.0	0.5	12
Asparagine	0.8	0.1	12
Aspartic acid	0.2	0.02	10
β-Alanine	0.22	0.03	14
Cysteic acid	0.2	0.05	25
Glutamic acid	0.1	0.01	10
Glutamine	0.4	0.05	12
Glycine	0.05	0.005	10
Histidine	7.5	1.5	20
Hydroxyproline	1.0	0.08	8
Leucine and isoleucine, 1:1	0.25	0.02	8
Lysine	1.5	0.18	12
Methionine sulphoxide	0.5	0.08	16
Phenylalanine	1.25	0.2	16
Proline	1.5	0.3	20
Serine	0.08	0.01	12
Taurine	0.2	0.03	15
Threonine	0.2	0.035	18
Tryptophan	2.0	0.4	20
Tyrosine	1.0	0.15	15
Valine	0.15	0.01	8

Results with Insect Blood

With the minimum quantities given in Table II, a quantitative estimation of the free amino acids contained in the blood of the last larval instar of *Galleria mellonella* (L.) was made. A qualitative analysis of the free amino acids contained in the larva of that species was published by Pratt (4). Samples of blood taken from several larvae are mixed together and diluted 1:10 with water by means of a diluting micropipette. This dilution is necessary because of the high concentration of free amino acids in insect blood. A decreasing gradient series of aliquots is measured and deposited on separate filter papers in the usual way. Subsequent to the separation in the solvents and development with ninhydrin, it is only necessary to note the amount of aliquot used on the chromatogram where a particular color spot was visible for the last time in the decreasing gradient series. With the minimum quantities recorded in Table II, it is easy to compute the concentration of each amino acid in the blood sample analyzed. The results obtained with insect blood are given in Table III.

TABLE III

FREE AMINO ACIDS CONTAINED IN THE BLOOD OF THE LAST LARVAL INSTAR OF *Galleria mellonella* (L).

Amino acid	Minimum quantity (Table II), μgm.	Minimum volume of blood showing amino acid, μl.	Concentration in blood, μgm./100 μl.	Amino nitrogen, mgm./100 ml.
Alanine	0.06	0.05	120	18.84
α-Amino-*n*-butyric acid	0.12	30	0.4	0.05
Arginine	4.0	6	67	5.38
Asparagine	0.8	4.5	18	1.91
Aspartic acid	0.2	0.25	80	16.84
β-Alanine	0.22	2.0	11	0.0
Cysteic acid	0.2	50	0·4	0.03
Glutamic acid	0.1	0.05	200	19.04
Glutamine	0.4	0.05	800	76.17
Glycine	0.05	0.3	17	3.01
Histidine	7.5	5	150	13.54
Leucines	0.25	0.25	100	10.68
Lysine	1.5	2.5	60	5.98
Methionine sulphoxide	0.5	1.0	50	4.75
Proline	1.5	0.5	300	36.5
Serine	0.08	0.2	40	5.33
Threonine	0.2	0.8	25	2.94
Tryptophan	2.0	55	3.7	0.23
Tyrosine	1.0	1.5	67	5.18
Valine	0.15	0.2	75	8.97
			2184.5	235.37

Discussion

Pratt (4), with the method of Van Slyke *et al.* for the quantitative determination of free amino acids by titration of the carbon dioxide formed in the reaction with ninhydrin (7), found 199.4 mgm.% of amino nitrogen in the

blood of the larvae of *Galleria mellonella* (L.). The results given in Table III for total amino acids show 235.37 mgm.% of amino nitrogen as calculated from the data on pure amino acids published by Van Slyke *et al.* (6). These results agree within 15.3% of Pratt's determinations, which is satisfactory considering that one is dealing with biological material.

In the application of the above described method, only a trace of each amino acid is required for the quantitative estimation. The volume and the concentration of the aliquots placed on the papers must be small, and interfering substances contained in biological material are therefore present only in negligible amounts. Quantitative procedures such as alcohol extraction, protein precipitation, etc., often necessary prior to the chromatographic separation of large samples, are eliminated in most cases when using the present ultramicromethod.

In order to obtain fairly accurate results, it is always necessary to proceed with a decreasing gradient series of aliquots from a sample. In such a series, each amino acid must be present in slightly decreasing amounts from a level above the minimum quantity to one just below. This is readily achieved by first chromatographing a few aliquots of widely different amounts of the unknown sample in order to determine at what range one must prepare an extensive gradient series for the quantitative estimation.

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AIR-MASS CLIMATOLOGY OF ONTARIO NORTH OF LAKE HURON AND LAKE SUPERIOR BEFORE OUTBREAKS OF THE SPRUCE BUDWORM, *CHORISTONEURA FUMIFERANA* (CLEM.), AND THE FOREST TENT CATERPILLAR, *MALACOSOMA DISSTRIA* HBN. (LEPIDOPTERA: TORTRICIDAE; LASIOCAMPIDAE)[1]

By W. G. Wellington[2]

Abstract

Previous work showed that ideal physical conditions for the development of the spruce budworm, *Choristoneura fumiferana* (Clem.), occur when the weather is relatively dry and clear. These conditions have tended to occur in summers when the annual number of cyclonic centers passing over the area was declining, and have preceded past outbreaks. On the other hand, the physical requirements of the forest tent caterpillar, *Malacosoma disstria* Hbn., include warm, humid, cloudy weather during much of the larval stage, and outbreaks of this species in Ontario have begun after an increase in the annual number of cyclonic passages. While the annual number of cyclonic passages is declining in periods before spruce budworm outbreaks in northern Ontario, the number of these passages in the summer months falls below average. Furthermore, the majority of the centers that do pass in these months contain air masses of polar continental or maritime origin. The more humid southwestern air masses are usually barred from the area by a southward shift of the whole circulation pattern. This situation is reversed in periods before *M. disstria* outbreaks. While the annual number of passages is increasing, the number occurring in the summer months is above average, as is the proportion of southwestern air masses occurring in these months. Northern and western air masses are usually active farther north, owing to a northward shift of the whole circulation pattern.

Introduction

In a previous paper (4), attention was directed to various physical and biological phenomena that consistently preceded outbreaks of the spruce budworm, *Choristoneura fumiferana* (Clem.), in northeastern North America. Among the physical indicators of particular interest were the observed decline in the annual number of centers of low atmospheric pressure which passed over an area prior to the beginning of an outbreak, and the reduced precipitation during June and July. It was also pointed out that many of the spruce budworm outbreaks in Ontario were preceded by infestations of the forest tent caterpillar, *Malacosoma disstria* Hbn., and that the major outbreaks of this species tended to begin after a period of increase in the annual number of cyclonic passages over an area.

When the earlier contribution (4) was prepared, the physical requirements of the spruce budworm were known in some detail, but those of the tent caterpillar were not. Additional information on the requirements of *M. disstria* is now available (3). Furthermore, additional meteorological data have made it possible to derive some quantitative expressions of changes in different types of air masses occurring before the different outbreaks. This permits a

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much sounder climatological presentation than one based simply on changes in annual numbers of cyclonic passages. The present paper reports the results of these studies. The analysis has been confined to the region bordering on and extending northward about 100 miles from the northern shores of Lake Huron and Lake Superior.

A Note on Air Masses

A brief discussion of the concept of air masses would be helpful at this point. An air mass is a portion of the atmosphere which is relatively uniform in its horizontal distribution of humidity and temperature and their rates of change with height. The air mass may be more than 1000 miles in horizontal extent, and it acquires its uniform properties by staying over a relatively uniform part of the earth's surface for a period sufficient to produce equilibrium between the surface and the lower levels of the air. When the air mass moves from such a source region, it carries with it characteristic properties, but these become more or less modified, depending upon the rate at which the air moves over new and different surfaces. Since it carries representative properties, modified though these may be, it contains a specific range of weather, so that it is permissible to refer to "air-mass weather".

This type of weather is important, but there is also another type that should be noted. This is frontal weather, which is associated with the zone of discontinuity formed between two air masses of different properties when they come in contact. Frontal weather produces not only more marked, but also more transient, changes in the observed weather of a district.

Portions of the earth's surface uniform enough to serve as source regions are mostly maritime. Land sources are confined to polar or desert regions, or, in summer, to relatively uniform northern forest or prairie. It is customary to assign code letters to air masses from various source regions. Thus, air from a polar source is designated P, and that from a tropical source, T. Further information on the maritime or continental origin of the air is given by placing the appropriate code letter in front of the designator, e.g., mT, cT; mP, cP. Additional subscripts may be added, but these are the only ones of present concern.

Of the several types of air masses which invade or originate in North America, three are of particular importance to this study. The first is polar continental (cP) air, which has a winter source at high latitudes, and moves from there chiefly in the form of areas of high pressure (anticyclones), although occasional cyclones appear. In summer more cyclones are produced, some at high latitudes, and many farther south over the plains. The stability of this air varies, but the air is frequently dry enough so that even strong convection *within* the air mass produces only scattered or broken cloud.

The second type is polar maritime (mP) air, and the air which enters Ontario is almost entirely of Pacific origin. It is derived from sources in the North Pacific, but it reaches the west coast after trajectories which vary considerably from north to south. If this air crosses the western coast of the

continent near or north of the international boundary, it becomes dry enough in crossing the main ridge of the mountains to be quite similar in this respect to cP air. If it enters first by way of the southwestern coast, and particularly if, thereafter, it has a trajectory over the southwestern area of the United States, it may become quite moist and act like modified mT air as it returns northward towards the Great Lakes.

The third type is tropical maritime (mT) air, largely derived from or near the Gulf of Mexico. The air is warm and moist, often excessively so. The country north and west of Lake Superior experiences only intermittent invasions by this type of air, but mP air of the southern type noted previously, or Lows forming in the Great Basin and moving near the Gulf prior to turning northward, usually act in its place. Typical mT air becomes more and more important to the southeast.

This description has been greatly oversimplified, but it contains the salient points necessary for an understanding of what follows. No gross errors will be made if it is assumed that air entering the country north of the two Great Lakes from a northerly or westerly direction will be relatively dry, whereas air entering from the west-southwest, southwest, or south will be increasingly moist, and usually warmer.

Physical Requirements of the Insects

The comments in this section summarize the observed effects of weather upon the active stages of the two species. The spruce budworm has been dealt with more fully in the previous publication (4) and the references cited therein give additional details. The insect's requirements may be summarized here by noting that any air masses that produce clear or only partly clouded skies during the summer months, particularly with a diminished intensity of precipitation, are considered to provide ideal conditions for development. Conversely, air masses or increased frontal activity producing densely overcast skies or frequent precipitation are less satisfactory.

Many of the requirements of the forest tent caterpillar during its active larval stage have proved to be opposite to those of the spruce budworm. Unlike the latter, *M. disstria* feeds exposed to the atmosphere. Furthermore, it is the only species of its genus on this continent that does not construct protective communal tents to which individuals may return at intervals. These points appear to have some bearing upon its requirements.

Although the larvae of both species are inactivated by continuing rain, *M. disstria* requires moist air to carry out its activities efficiently. An impressive example of this need was observed during laboratory tests of larval reactions to point sources of light. During one series of tests at a temperature of 21°C. and a relative humidity near 45%, larvae were so sluggish that they seldom moved unless prodded, so they did little but face the light. A second series of tests was made at the same temperature, but the humidity was increased to 80%. In these tests, larvae moved rapidly and incessantly, and orientated

with precision. Chilling by evaporation is indicated in the first instance, but the example shows the need for moist air even at moderate temperatures.

During the early instars, a relatively clear sky is necessary during cool days, for dense clouds diminish radiant heating and, consequently, the time available for feeding is shortened. On the other hand, solar heating is more intense during the greater part of the larval stage in late May and June. During this period, larvae exposed to the resulting high temperatures spend more time in moving to shaded sites than they do in feeding. Furthermore, during the clear nights that follow such days, the temperature drops quickly enough so that nocturnal feeding is curtailed. Consequently, warm, humid, partly cloudy weather tends to accelerate development during the greater part of the larval stage, and any air masses which produce this weather provide ideal developmental conditions.

Materials and Methods

Each issue of the *Monthly Weather Review* of the United States Weather Bureau contains a map showing the paths of all centers of low pressure recorded during the month. Kullmer's (2) grid system of rectangles, each five degrees of longitude by two and one-half degrees of latitude, was used as before to record the number of centers passing over a particular area. However, instead of recording the centers simply on an annual basis, monthly totals were taken, and these were further subdivided into mT, mP, and cP air masses.

It is important to note that this treatment introduces simplifications that have certain dangers. The first simplification is that the centers passing near, but not over, an area are lost. This was criticized with some justice some years ago (1) and an alternative method was suggested, but the errors introduced may be disregarded for the present purposes because considerable correspondence was found between results obtained by the two methods. The important point is that the numbers counted are always small in the less accurate method.

Classification of cyclonic centers by source regions introduces a second simplification that can introduce more serious errors. It must be kept in mind that, soon after these centers leave their points of origin, they frequently enter into complex associations with other types of air, in which frontal systems develop. The cyclones then may be composed of at least three different air masses, only one of which may be referable to the source region. The argument in favor of classification by source regions despite this ultimate composite structure of a center is based on the following points: (*a*) the original source region air mass is contained in the structure; (*b*) the weather associated with part of the period of passage of the center is usually typical of the source air mass; and (*c*) the circulation patterns referred to later reinforce the conclusions based on data classified by sources. Consequently, the method yields results that can be cross-checked.

Tropical air masses were recorded in two subgroups. The first contained mT air of Atlantic origin. The second was a mixed group of more western air masses that included mP air that entered California or southern Oregon

directly, and other air masses that had moved over the Great Basin or near the Gulf of Mexico prior to turning northeastward.

More northern mP air was recorded as such, even when it moved down via Alaska and the Yukon Territory. Polar continental air masses were recorded in two subgroups. One contained centers originating above latitude 60°N. The other contained those from the northern and central plains.

The published map, on which the tracks of centers are originally recorded, has varied considerably in area during the last few decades. Before 1930, little territory north of the latitude of 55° was included, and there have been intermittent improvements in the collection of far northern data since that time. Consequently, the years before 1930 were not used for source region classification because the earlier smaller map size made it difficult to judge the origins of some of the northern centers. This gave only a 20-year period, 1930-1949, for the extraction of mean values, a length of time ordinarily not sufficient for the establishment of trustworthy normals. Therefore, the data have not been subjected to tests of statistical significance, but it will be seen that the results match so well with those previously obtained by very different methods that their practical significance can be accepted.

When the necessary information had been compiled, it was examined in two principal ways. First, the total number of centers of all types passing over an area during a year was recorded, and the number which occurred in any month was expressed as a percentage of the annual total. When each month was treated in this way, a graph was plotted to show the proportion of the annual cyclonic passages that occurred during each month. This was done first for the 20-year period to obtain average values, and then the data for the four-year periods preceding individual outbreaks were treated in the same way and compared with the 20-year values.

In the second method, which was somewhat similar, individual months replaced the year as the unit. Each type of air mass contributes a certain proportion of the total cyclonic passages in any month. Therefore, the numbers of centers contributed by each type of air mass were expressed as percentages of the monthly totals. As before, this was done first for the 20-year period, and then for the four-year periods preceding outbreaks. Some of the results obtained by these two methods are illustrated in Fig. 1.

The results obtained by these methods indicated a regular variation in the proportions of migratory air masses of different types that occurred before outbreaks of the two species. Therefore, the types of circulation which occurred during these preoutbreak periods were determined for selected months. The number of centers of all types passing through each rectangle of the grid during the month was entered in each corresponding rectangle on a base map, and isolines were drawn to determine the regions containing higher concentrations of passing centers. An arrow drawn along the middle of a zone of high concentration gave a rough indication of the average direction of cyclonic centers of one type.

Conclusions based solely on the isoline method are subject to errors if the finished map is not compared with the published map showing the original, scattered paths. When this is done, necessary adjustments to indicated directions can be made, and the final product is trustworthy for limited purposes. For instance, it illustrates the trajectories occurring during the month very well. It also shows the relative numbers of centers contributing to the types of circulation reasonably well. Since it is so simplified, it does not show the breadth of the area spanned by all the paths of centers of one type. Therefore, in interpreting the diagrams, it must be remembered that centers somewhat similar in direction to a generalized trajectory may have existed on either side of the path shown by an arrow. Samples of these maps are shown in Figs. 2 and 3, and are discussed in the following section.

Results

Fig. 1 contains samples of graphs constructed by treating either cyclonic centers or specific types of air masses as percentages of annual or monthly

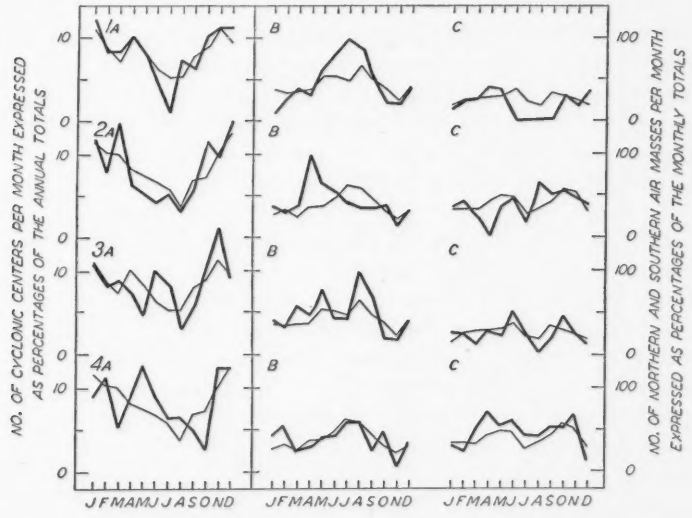

FIG. 1. The deviations of the average numbers of cyclonic centers or specific air masses for four-year periods preceding given outbreaks from the average numbers for the period, 1930-1949. Light lines, the values for the period, 1930-1949; heavy lines, the four-year groups. Diagram A in each numbered series shows centers of all types considered monthly; B shows air masses of northern continental types, and C shows southern and southwestern air masses, usually maritime in origin. Spruce budworm, Series 1: Lake Nipigon area, 1936-1939; Series 2: Mississagi area, 1931-1934. Forest tent caterpillar, Series 3: Lake Nipigon area, 1932-1935; Series 4: Lake Huron North Shore area, 1944-1947.

totals of cyclonic passages. The light lines in all the graphs show the average values based on the period, 1930-1949, and the heavy lines refer to averages of specific four-year periods immediately preceding particular outbreaks.

Series 1 and 2 refer to periods before spruce budworm outbreaks in the Lake Nipigon and Mississagi areas of Ontario, respectively. Series 3 and 4 refer to periods preceding forest tent caterpillar outbreaks in the Lake Nipigon and North Shore of Lake Huron areas, respectively.

Diagram 1, A shows the monthly totals of cyclones of all types for the period, 1936-1939, compared with the 20-year averages. The significant feature shown is the deficit in numbers during the summer period. Diagrams 1, B and 1, C show that the bulk of the air masses that occurred during this summer deficit consisted of northern continental types originating in the polar regions or in the northern and central plains, whereas there was a marked deficit in southern and southwestern air masses.

Diagram 2, A shows that the monthly totals for the period 1931-1934, in the Mississagi area, were deficient during the spring and early summer, and less so during July, compared with the 20-year averages. Diagrams 2, B and 2, C show there was an excess of northern air masses and a deficit in southern types in the spring. By June, the excess of northern air masses was slight, and a deficit appeared during July. In both these months, there was a minor deficit in southern air masses. Polar maritime air masses are not illustrated, but they provide the significant link in this series. Over the four-year period, invasions by mP air were below average from January to June, but, in July, when there was a lack of purely polar continental air, there was a series of invasions by mP air with westerly or west-northwesterly trajectories. These centers accounted for 40% of the total number of cyclonic passages for that month, and their total should be added to that of the northern continental types.

During the first half of the 1930 decade, outbreaks of the forest tent caterpillar began to occur in northwestern Ontario. The first infestations began far to the west of Lake Nipigon but, eventually, about 1936, significant increases in population seem to have begun near there. Hence, the diagrams of Series 3 are of considerable interest. They show conditions occurring during 1932-1935, and may be used not only for the peripheral infestations of tent caterpillars that appeared in the area shortly thereafter, but also for comparison with the later conditions during 1936-1939 (Series 1), which preceded the major spruce budworm outbreak in the area.

Diagram 3, A, compared with 1, A, shows there was an excess of cyclonic centers in June and July during the earlier period, instead of a deficit. Diagrams 3, B and 3, C show that, during this summer period, there was a slight deficit in northern air masses and a slight excess of southern air masses. These differences are interesting when they are considered in terms of the physical requirements of the spruce budworm. Furthermore, although this area must be considered to have been a peripheral zone of forest tent caterpillar activity instead of a primary focus, the trends shown are interesting when they are compared with the diagrams of Series 4.

Diagram 4, A shows a varying excess of cyclones from May to August during 1944-1947, compared with the 20-year averages. The graphs of this

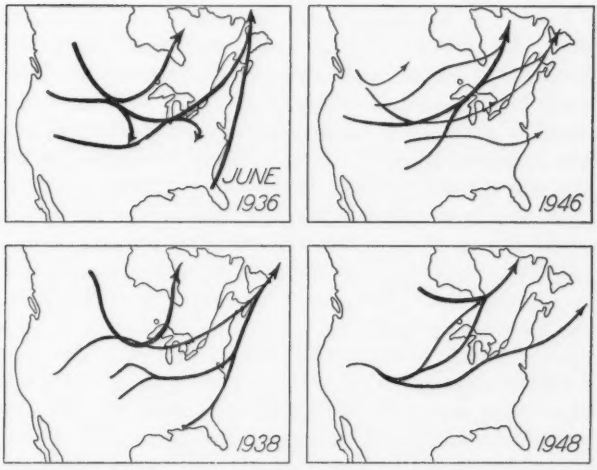

FIG. 2. Circulation diagrams for the month of June during the years 1936, 1938, 1946, and 1948. The diagrams for the first two years illustrate the southward shift of the complex of trajectories during the 1936-1939 period preceding spruce budworm outbreaks. Note the northerly trajectories of centers most influencing the region north of Lake Superior and Lake Huron. The last two diagrams show the northward movement of the complex of trajectories and the greater influence of southwestern air masses upon the country north of the Great Lakes in the period preceding outbreaks of the forest tent caterpillar.

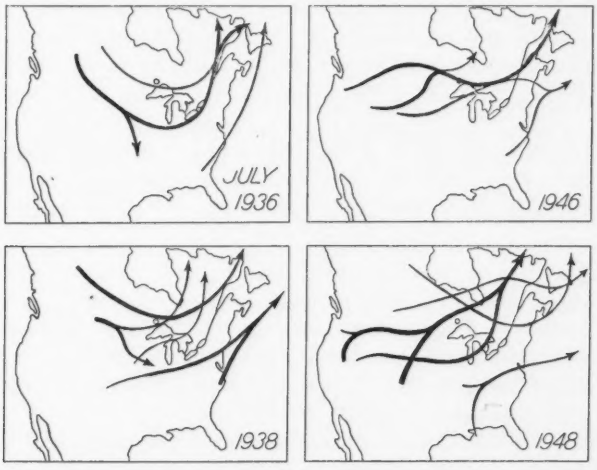

FIG. 3. Circulation diagrams for the month of July during the years 1936, 1938, 1946, and 1948. The diagrams for the first two years show a marked southward shift of the circulation pattern and an increased influence of northern air masses on the country north of the Great Lakes during the 1936-1939 period preceding spruce budworm outbreaks. The diagrams for the last two years show a marked northward shift of the circulation pattern and a strong influence of more southwestern air masses upon the country around the Great Lakes in the period preceding outbreaks of the forest tent caterpillar.

series refer to conditions along the northern shore of Lake Huron, but they also may be compared with those of Series 2, the Mississagi area to the north, since it is impossible to separate the two areas adequately on maps of the scale used for the published paths. Diagrams 4, B and 4, C show a very minor deficit in northern continental air masses during the summer, and a marked excess of southwestern types. Polar maritime air was deficient in amount until September, and was entirely absent during June, July, and August, so that there was no reinforcement of the effects of cP air during those months.

Among other things, these results indicated air movement predominantly from the north and west during summers preceding spruce budworm outbreaks, and from the southwest during summers preceding tent caterpillar outbreaks in the area north of the Great Lakes. The circulation diagrams constructed as described in the preceding section show these changes very well. Fig. 2 shows four diagrams for the month of June, and Fig. 3 illustrates four for July. It is unwise to average individual months for short spans of years with techniques in their present state. Therefore, only months in individual years are shown in the diagrams. The years 1936 and 1938 serve for the period, 1936-1939, which preceded several spruce budworm outbreaks. The years 1946 and 1948 show conditions 10 years later, and serve for the period prior to and during the beginning of several forest tent caterpillar outbreaks that have occurred west of Lake Superior and along the Canadian shores of Lake Huron.

The years 1936 and 1938 show that there was a strong northerly component in the trajectories of centers influencing the area north of the Great Lakes. Moreover, the whole complex of trajectories was shifted southward. The years 1946 and 1948 show that the more northern air masses were absent from the region of the Great Lakes. In fact, many of them were active above the top of the grid, which was drawn only as far as latitude 55°N. In addition to this northward shift, it is clear that there was an increase in activity among the more southern types, and the dominant trajectories in the area of interest were from the west-southwest or the southwest.

Discussion

The examples illustrated in the results, together with those presented previously (4), provide cumulative evidence for distinct climatic sequences in the complex of events preceding outbreaks of the two species. Consequently, the relation of these climatic sequences to the development of the outbreaks can be stated with some confidence.

If changing annual numbers of the migratory cyclonic centers are considered alone, major outbreaks of the forest tent caterpillar in Ontario have tended to appear as these annual numbers rise towards a maximum. The Ontario outbreaks during the 1930 and 1940 decades began in different areas at different times in relation to the year of maximum number of cyclones, i.e., from three years before the maximum to one year after it. Conversely, spruce budworm outbreaks have started after the annual numbers of centers have begun to

decline from a maximum. Starting dates for these outbreaks have been distributed from three to six years after the year of the maximum number of cyclonic centers. For either species, part of the variation in time may be due to difficulties in establishing the real beginning of an outbreak. In any event, the implication is that physical conditions favorable to increases in populations of the forest tent caterpillar begin to occur as the numbers of cyclones rise from a minimum, and that these conditions continue to recur up to the early years of an outbreak. On the other hand, conditions favorable to increases in populations of the spruce budworm begin to occur soon after cyclones start to decline in numbers, and the favorable trend is intensified through the early years of an outbreak.

If the proportions of cyclonic passages in different seasons of the year are considered, there is a noticeable deficit in summer during groups of years characterized by declining annual numbers of cyclones, i.e., those years preceding spruce budworm outbreaks. In addition, if the proportions of different air masses contributing to the monthly totals of cyclones are considered, the period of summer deficit is further characterized by a decreased proportion of humid air masses from southern and southwestern areas and an increased proportion of drier air masses from the north and west. These relationships are reversed in groups of years preceding tent caterpillar outbreaks.

Finally, if the average trajectories during the two types of periods are examined, periods preceding spruce budworm outbreaks are characterized by a general southward displacement of the circulation complex during June and July, so that cP and mP air must predominate in the areas of incipient outbreaks, whereas mT air and its subsidiaries are absent or effectively barred. Conversely, during periods preceding increases in populations of *M. disstria*, there is a northward displacement of the complex and more invasions by air masses from southern quadrants occur. Northern types of air masses are not necessarily absent from the continent but, when present, they are usually active farther north.

There is a point concerning anticyclonic activity. It was noted earlier that much of the cP air occurs in the form of anticyclones. A northerly circulation would also favor invasions by these pressure systems, so that their effects would be added to those of the northern cyclones. In fact, it would be possible to present results in terms of movements of anticyclones instead of in terms of movements of cyclones, but the latter method enables one to transfer from air masses to frontal activity more readily.

This leads to the point that frontal weather cannot be entirely neglected, although only air-mass weather has been considered in detail here. It is clear that the frequency of occurrence of frontal systems is related to the total numbers of passing cyclones. Thus, when increased numbers of cyclones are associated with more frequent invasions of the areas of interest by humid air masses, the effects of this air-mass weather are enhanced by an increased number of frontal passages. On the other hand, when decreased numbers of passing cyclones are associated with a preponderance of invasions by drier air

masses in summer, the effects of the greater stability of this air-mass weather are enhanced by the accompanying decrease in number of frontal passages.

Whichever way the general problem is approached, a particular end result from the standpoint of each species consistently recurs. The spruce budworm populations are favored by recurring dry summer conditions and those of the forest tent caterpillar are favored by humid conditions. Each of these basic conditions is in turn associated with particular cloud types and amounts, which also affect the amount of solar heat that can penetrate to the niches occupied by the species. The various large-scale atmospheric processes studied here favor the production of these different physical conditions during different periods in the same broad areas.

The continued emphasis upon events occurring during the late spring and summer periods of activity of the two species, particularly during June and July, does not mean that no importance is attached to weather occurring during the remainder of the year. Other sensitive stages in the life cycles occur at other seasons, and the weather at these times may be of considerable importance. Nevertheless, the coincidence of more consistently recurring weather patterns with the period of activity is very marked. The relations are clear enough so that a system of forecasting increases to outbreak level, based upon events occurring during the period of activity, should be free from serious errors. Therefore, until the need for greatly increased accuracy is clear, this restricted period alone will be used.

It is worth noting that no climatological system of forecasting population changes will be very accurate until more trustworthy long-range forecasts of weather or climatic trends are available from official meteorological services. Few such forecasts exist at present, but one may look forward to useful developments in this field. Since the official systems that may appear in the future must put some weight on changes in the general circulation, the work with the insects has now been brought to the point where it can be quickly fitted into the framework of these systems when they appear.

Meanwhile, inspection of the limited climatic data available suggests that some increases in populations of the spruce budworm should begin to occur during the next few years in the 100-mile belt lying north of the two lakes and extending westward to the Manitoba boundary. The emphasis here is on population *increases*, since it is probable that no increase to *outbreak* level can occur in areas where there is now a lack of suitable hosts due to extensive damage from the last outbreaks. However, careful sampling should show minor rises, and, in areas that were relatively undamaged during the last outbreaks, increases to minor outbreak level may occur.

In the belt considered, annual numbers of migratory cyclones reached their peak in most areas between 1947 and 1949, so that the decline in their numbers has already begun. Modifications in the circulation are beginning to produce easily noticeable differences from the unsettled weather that was common around the time of the maximum. The earliest year in which a noticeable increase in the spruce budworm might have been expected on these

grounds was 1951, and there are indications from Forest Insect Survey samples taken from northwestern Ontario that small rises did occur then. The years in which initial population increases should become more noticeable are 1952-1955, and, in suitable foci, one would expect development to the minor outbreak level. The next minimum in the annual numbers of passing cyclones should occur about 1956 or 1957.

There is one additional point in connection with the use of these physical phenomena for biological forecasting that may have some influence on the trends suggested above. Throughout this paper, the emphasis has been on changes in the numbers of cyclonic centers or types of air masses that pass over a particular area. It has been shown that changes in the general circulation account for part of the changes in the number of passages, but little stress has been placed on the varying rate of production of the pressure systems. During the course of the present work, it became very clear that there is an orderly fluctuation in the numbers of cyclones and anticyclones that occur over the whole continent and its adjacent oceans. The details will be published elsewhere, but certain points are summarized here because of the bearing they may have on biological problems. Numbers of anticyclones usually vary in the same direction as numbers of cyclones, so the summary will be confined to the latter.

Numbers of cyclones occurring over the whole continent were counted for each month for the period, 1910-1950, without regard for their sources or subsequent paths. The total annual number of primary plus secondary centers varied during the period from a low of 107 to a high of 613, and the possible impact of this great change in storminess upon organisms of many kinds is thought-provoking. Only three minima and three maxima have occurred during the span of years investigated, so that it would be premature to call the observed fluctuations cycles. The intervals between maxima have varied considerably, but the interval between absolute minima has been 15 years in each instance, e.g., 1913, 1928, 1943.

The first and last of these minima were associated with the decades in which there occurred two groups of spruce budworm outbreaks in northeastern North America violent enough to attract special attention. The interesting point is that the minimal numbers of cyclones occurring over the whole map in 1913 and 1943 were 115 and 107, respectively, whereas the number in 1928 was 192. It seems possible that reduced cyclonic activity could exaggerate the deficiencies due to changes in atmospheric circulation that are observed in particular areas, and so help to produce more violent outbreaks at such times, where the forest is suitable. To continue this line of speculation, it seems possible that very low minima would be more effective than those that were only moderately low. Unfortunately, there is as yet no way to judge just how low the next minimum (1958?) will be. An additional point is that the periods of the two phenomena, total production of cyclones and passage of some of these over a restricted area, do not appear to be entirely in phase,

and this suggests that there could be either mutual reinforcement or, alternatively, partial neutralization of the effects of these two changes in numbers at different times. These comments are completely speculative at the present time, but they suggest avenues for future research.

The work reported here has been confined to a particular region of Ontario because there have been greater opportunities for first-hand observations of the effects of different air masses and changing circulation upon the weather and climate. Consequently, the conclusions should not be extended indiscriminately to such regions as the eastern or western coasts. The physical requirements of the species should be similar. Also, the range of weather producing the ideal conditions should be quite similar, if due allowance is made for the effects of topographic peculiarities. Moreover, previous work (4) showed that total numbers of cyclonic passages seem to have similar effects. However, it is not safe to assume that the same types of *air masses* will give the same effects, or will even be involved, particularly on the west coast. Therefore, additional air-mass analyses are required for eastern and western regions.

In the west, it would be useful to take into account the changes in the position of the California High, since its migrations exert a powerful influence. In the east, mT air of Atlantic origin should be more important among the complex of southern types, and it might also be profitable to examine more closely the apparent east–west shift that seems to occur in the complexes of trajectories illustrated, in addition to their more obvious north–south shift.

Conclusions

1. The physical requirements of the spruce budworm and the forest tent caterpillar during their spring and summer periods of activity show how weather and climate may influence the trends of their populations during pre-outbreak periods.

2. Ideal physical conditions for spruce budworm development occur when the air is relatively dry and clear. The best conditions for tent caterpillar development appear to be humid, partly cloudy, weather throughout the greater part of the larval stage, although direct sunshine is required in early larval life if the air is cool.

3. The required physical conditions for spruce budworm population increase tend to occur in northern Ontario when the annual number of cyclonic centers passing over an area is decreasing. During this time, the number of cyclonic passages in the late spring and summer is below average, and the majority of the air masses involved in passages during these seasons are dry. They are of polar continental or polar maritime origin, because a southward shift of the circulation pattern holds invasions of more southern air masses to a minimum.

4. The required physical conditions for forest tent caterpillar population increase begin to occur with increasing frequency as the annual number of passing cyclones rises to a maximum. During this period of increase in

cyclones, the number of passages during spring and summer is above average, and the majority of the air masses involved during these seasons are of southwestern or southern origin because there is a northward shift of the circulation pattern which moves the more northern air masses to higher latitudes.

5. These findings place the problem of forecasting population increases to possible outbreak levels on a meteorological basis that should fit into the techniques for long-range weather forecasting that may be developed by official weather services in the near future.

Acknowledgments

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MOVEMENT OF MATERIALS IN THE HYPOLIMNION OF A LAKE AS STUDIED BY THE ADDITION OF RADIOACTIVE PHOSPHORUS[1]

By J. A. McCarter,[2] F. R. Hayes,[3] L. H. Jodrey,[4] and M. L. Cameron[4]

Abstract

Approximately 100 millicuries of radioactive phosphorus in the form of potassium dihydrogen phosphate were introduced into the hypolimnion of a small acid-bog lake by causing a bottle containing a solution of the material to burst about 3 meters below the thermocline and about 1 meter above the bottom. The depth of water at the point of introduction of the bottle was 6 meters. After bursting the bottle the distribution of the radioactive phosphate through the waters of the lake was followed by withdrawing and analyzing samples of water from different localities and from different depths. Samples of ooze from the bottom of the lake were also taken for measurement of their content of radioactive phosphorus. It was found that the radioactive phosphate moved through the hypolimnion in a lateral direction at a rate of some 3 meters per day. Although it moved laterally to the end of the lake (42 meters), the extent of its movement vertically appeared to be not greater than 2 or 3 meters, i.e., it scarcely penetrated the thermocline to a measurable extent. The radioactive phosphorus appeared early in the mud of the lake bottom, and its concentration there increased to such values as to suggest that a large part was removed by the mud, probably by a process of exchange of phosphate between mud and water.

Introduction

In an earlier paper (Coffin *et al.* (1)) an experiment was described in which radioactive phosphorus, P^{32}, was added to the surface of a small lake near Halifax, N.S. The lake is of acid-bog type, has an area of some 0.3 hectares (0.8 ac.), and reaches a depth of 7 meters. It has a definite thermocline and there is no oxygen in the depths. Details of its physical and botanical characteristics are given in the previous paper.

On June 17, 1949, shortly after dawn, approximately 100 millicuries of radiophosphorus were introduced in the deepest part of the lake, about one meter above the bottom. The procedure was as follows. On the evening of June 16, 4 gm. of potassium dihydrogen phosphate containing the active material, was placed in a wide-mouthed reagent bottle of 2045 ml. capacity, which was then filled with lake water. The bottle was stoppered with a plastic screw cap, through which were passed two wires, forming the contacts of a dynamite cap suspended inside the bottle. The holes for the wires were insulated against water leakage. Practically no air space was left in the bottle when it was closed. The bottle was lowered from a fixed raft to its position in the lake and left overnight for temperature equilibration. The next morning it was exploded by means of a dry cell on the raft. One or two small

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bubbles rose to the surface but, at the site, analyses for radiophosphorus were negative. Previous tests had shown that the effect of detonation was to disintegrate the bottle into thousands of pieces.

To simplify the collection of samples, a raft was set up over the deepest part of the lake and held in position by eight lines radiating to the shore. The positions of the lines are shown in Fig. 1, A which also gives the main contours of the lake. Each line was marked at 10 ft. intervals from the center to the shore, and samples were collected at various depths at these points. In order to prevent contamination of the lake surface with water from the depths, any excess in the sampling bottle was collected in a tub and carried ashore.

Bottles of water were brought to the laboratory, and radiophosphorus estimated as previously described. One counter and scaler was used for all determinations. Results were expressed as counts per 100 ml. of water. Samples of mud and aquatic organisms were examined from time to time, results being converted to counts per 100 gm. wet weight. The volumes of water which were evaporated for counting varied from 100 to 500 ml. depending on the amount of activity. The weight of mud or living organisms counted was of the order of 0.1 gm. dry weight. About 500 samples of water were tested in all, and about 125 samples of mud, together with a few counts on organisms. On the first day about 137 samples of water were collected.

A disadvantage in the procedure is the necessary time-lapse of several hours between collection of a water sample and the result of a count on it. Hence in early days the collector does not know at what stations samples are likely to be active. Ideally, tests would be made by a dipping counter and read from the boat. Unfortunately however, phosphorus in solution is adsorbed on the surface of the counter giving a "memory" effect which makes the instrument useless.

We have not been able to hit upon any graphical method of presenting intelligibly all the water analyses. Fig. 1, B, however, does give the general picture of movements in the hypolimnion. The contours on the figure show the limits at which radiophosphorus was detected at the times shown.

Immediately after the explosion the 10 ft. circle was tested at all depths and results were negative. The lake surface above the center of explosion was also negative. No tests were made at the supposed center of activity. Six hours later samples were taken at a depth of 12 ft. and at radii of 6, 8, and 10 ft. The contour marked "1" in Fig. 1, B is based on this reading.

Contours for later dates are based on series of tests at 10 ft. circumferences and 3 ft. depth intervals. It is evident that by 14 days the radiophosphorus had reached the shallow area by the outlet stream of the lake. Calculations of the rate of movement of water in a southerly direction indicate a speed in the hypolimnion of 3 ± 1 meters per day. The 6 ft. depth reading at Day 14 and near the outlet stream was higher than elsewhere in the lake at this depth suggesting that hypolimnetic water might have been pushing up towards the surface on reaching the shallows. By Day 32 the whole of the southern end of the hypolimnion exhibited activity. As to the northern end of the lake,

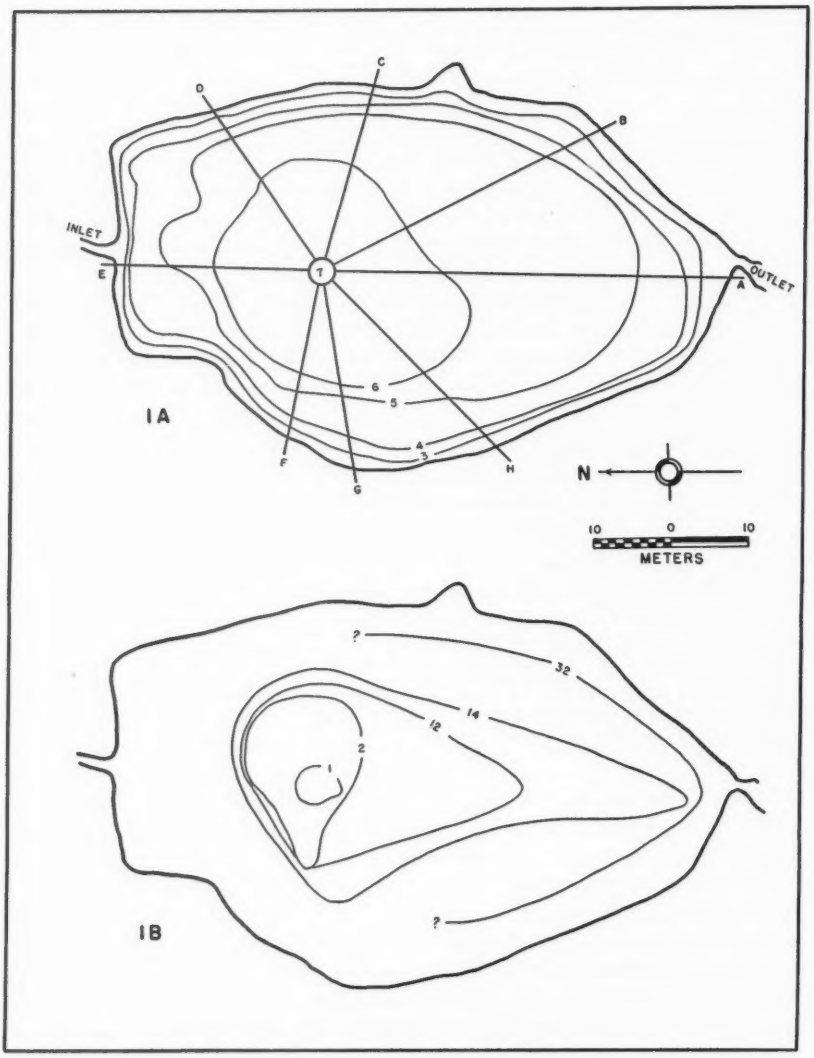

FIG. 1. A. Map of lake used in experiment. Depth contour lines are in meters as marked. The straight lines leading to letters at the lake periphery indicate positions of ropes leading from a raft and fastened ashore. It was along these ropes that observations were made at various depths. The raft was over the deepest part of the lake, approximately 7 meters. For a map showing botanical characteristics of the lake, see Coffin *et al.* (1).

B. Outline map of lake to show how far the added radiophosphorus had penetrated horizontally at various days after its introduction below the raft. The date of introduction is called Day 1. By Day 51 (not shown in figure), there was a penetration of radiophosphorus to shore in all directions of the hypolimnion.

information on Day 32 is incomplete. Observations at earlier dates suggested that the movement of water in this direction was slower than to the south.

Activity in the epilimnion was at no time comparable with hypolimnetic counts. For example on Day 2, the following counts were read on Rope *D* at 20 ft. radius:

Depth, ft.	Depth, meters	Counts per 100 ml. water
3	0.91	0
6	1.83	2
9	2.74	32
12	3.66	114
15	4.57	333
18	5.49	1823

The bottom of the thermocline would be at about 2 m. By Day 14 there was evidence of some small penetration to the surface, but the temperature barrier was still very effective.

Evidence for penetration of radiophosphorus to the surface is also available from analyses of living organisms. As previously shown, these concentrate the activity to a level 100 to 40,000 times that of the water surrounding them. Hence a test on an organism (as compared to water) has the same effect as a large increase in the sensitivity of the measuring instrument. It is not surprising therefore that organisms at the surface did exhibit activity expressed as follows in counts per minute per 100 gm. wet weight.

Organism	Day	Count
Sponge	12	7720
Sphagnum	12	5598
Utricularia	12	31,560
Algae	14	48,950
Zooplankton	32	Dawn 287,000
		Dusk 254,500

It was thought that some exchange between the surface and deeper layers of the lake might be caused by vertical migration of zooplankton, which occurs in many lakes in relation to light. It was known from the earlier experiment on this lake, that zooplankton exhibit a very rapid uptake of added phosphorus. Thus if the organisms were in the hypolimnion overnight the radiophosphorus in them might be expected to increase, and therefore a difference in activity might be looked for between samples collected at dawn and at dusk. The last lines of the table show the results of such counts. There is no significant difference between them, hence no evidence to suggest that these animals provide a mixing mechanism by vertical migration. Whether they would do so in a more transparent lake whose depths contained an adequate supply of oxygen is another question.

Samples of mud were collected at intervals with an Ekman dredge. The surface layer was scraped off for measurement of activity. Laboratory observations made since the sampling was done have shown that the active layer of mud is very thin, less than 1 mm. so that for counts of high accuracy great care would be necessary in order to take only surface ooze as a sample. This was not known at the time, and doubtless there was a variable dilution of active material by mud from a few millimeters below the surface. Because of this dilution it is not possible to attempt to account for all the added radiophosphorus by summing up the mud and water values.

The results of analysis of about 125 mud samples taken from all directions and distances from the center are shown in Fig. 2. Points up to 40 ft. (12 meters) are based usually on five or six samples, those farther out on fewer. As an example of the level of variability of results, take the data for Aug. 6, which was Day 51. Counts are given in thousands per hundred grams of dried mud; for wet mud divide by 10.

	Distances from center in feet							
Rope	10	30	50	70	90	110	130	150
A	141	53	14	14	43	21	0	0
B	130	72	8	0	10	218		
C	301	49	4					
D	346	113	20					
E	392	713						
F	541							
G	956							
H	598	133	109	33				

The two lines have been placed on Fig. 2 to suggest (*a*) that the levels of activity were not yet maximal in the mud by Day 6 and (*b*) that those close to the center had by Day 13 virtually approached their maximum, since no further gain was later evident. As to observations beyond 12 meters, the two series on Day 32 and Day 51 are not discernibly different.

From other experiments (2), it has been concluded that there is a continuous exchange of phosphorus taking place between water and the mud surface. At the beginning of the present experiment, all the radiophosphorus would be near the explosion center and a good deal would pass into the mud, yielding the high counts observed at Day 6. As time passed and the active material became dispersed throughout the hypolimnion there would be less to be exchanged near the center, so that the mud there would become stabilized with respect to radioactive material. At the same time an exchange would be progressively set up with mud more distant from the center. When equilibrium was reached, most of the radioactive material would be found in the mud surface. Because mud is the great reservoir of phosphorus, the few atoms

of radiophosphorus are like needles in a haystack. Their return to the water would be very slow. Thus no decline in mud radiophosphorus was detected in later observations near the center.

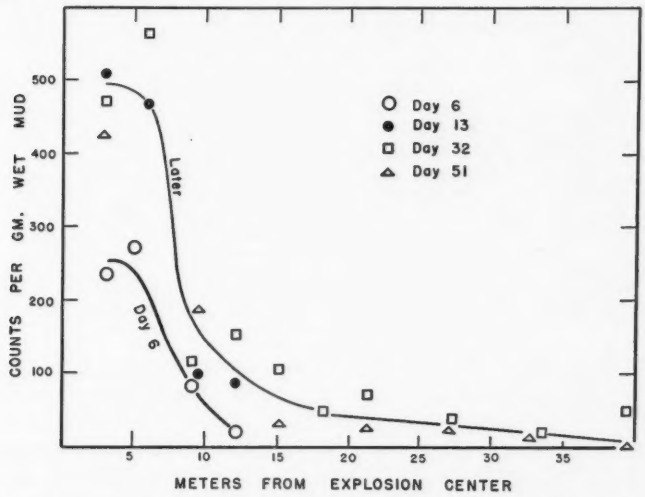

FIG. 2. Counts of radiophosphorus per gram of surface mud, at various distances from the explosion and at various dates. Points represent averages of values in different directions from the center. It appears that an area of 5 or more meters radius is uniformly active, while more distant parts show a sharp decline. It also appears that on Day 6, mud counts were not yet maximal, but by Day 13 they were, since later observations showed no increase in counts. Obviously the uptake by the mud would taper off as the level of radiophosphorus declined in the water.

Acknowledgments

It is a pleasure to acknowledge the assistance of Mr. James Lewin and Miss Shirley Mason.

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VASCULARITY OF THE HYPOPHYSIS OF LOWER VERTEBRATES

THE PAINTED TURTLE, *CHRYSEMYS PICTA MARGINATA* AGASSIZ[1]

By Stanley James Taylor

Abstract

Measurements were made on the diameter and length of the capillaries of the turtle hypophysis. The volume and surface of vessels were calculated and compared with those of the cat, frog, and salamander. Only the anterior lobe showed vessels which were significantly wider than in the adjacent brain tissue. The surface area of blood in the anterior and tuberal lobes is very extensive, 17 sq. mm. to 18 sq. mm. for 1 cu. mm. of fresh tissue, and is between two and three times as great as in the other lobes and in the richest centers of the brain. The vascular surface in each lobe, except the intermediate one, is lower than, but shows similar variation to, that of the cat. The pars nervosa is supplied by vessels between it and the pars intermedia as in the salamander and is unlike that of the frog or cat with numerous vessels penetrating its substance.

The structure of the hypophysis of various reptiles has been described by de Beer (1926 (2)), Altland (1939 (1)), Poris and Charipper (1938 (30)), Gorbman (1941 (17)), and Poris (1941 (29)). De Beer stated that in the tortoise "the pars anterior (distalis) is very loosely attached to the posteriorly projected infundibular stalk and is a large compact mass consisting of nests of cells placed posteroventrally. Separated from it by the more or less obliterated infundibular cavity is the pars intermedia to which part is also closely attached the pars nervosa. Anterior to the pars intermedia and in contact with the tuber cinereum is the pars tuberalis, a lobe of very small extent in this class." Fig. 1 (after de Beer),[2] shows this condition.

Since the blood supply of a gland of internal secretion is a matter of primary importance, some attempts have been made to express this quantitatively. Stevens (1937 (37)) concluded that the anterior lobe of the cat is six times as richly supplied with blood as the posterior (neural) lobe, the latter having the same vascularity as the richest center of the brain, while the intermediate lobe was poorly supplied and the tuberal lobe was richly supplied with capillaries. In contrast to this condition, Craigie and the author (1938 (38)) found that in the frog the pars nervosa was the most vascular lobe followed in order by the anterior, tuberal, and intermediate lobes. In *Ambystoma* Craigie (1938 (11)) found that the anterior lobe was five times as rich in capillaries as the intermediate lobe, and that the pars nervosa and pars tuberalis were provided only with a superficial net.

These quantitative studies show that in the mammal the pars anterior is much greater in capillary richness than in amphibians (Fig. 4). De Beer stated also that the anterior lobe increases in importance and the pars intermedia decreases as one ascends the evolutionary scale. Moreover the pars

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Contribution from the Department of Zoology, University of Toronto, Toronto, Ont.

[2] *With kind permission of publishers Oliver and Boyd, Edinburgh, Scotland.*

intermedia shows a poorer vascularity in mammals than in amphibians. If vascularity is one index of functional activity, the comparative capillary richness of the pars anterior in the cat and the poverty of the pars intermedia

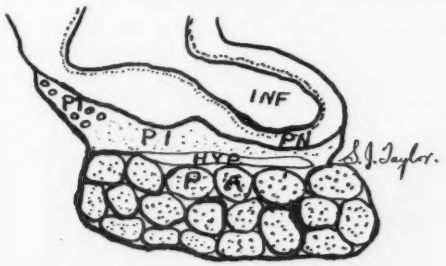

FIG. 1. Sagittal section of turtle hypophysis, after de Beer (1926 (2)). HYP, hypophysial cavity; INF, infundibulum; PA, pars anterior; PI, pars intermedia; PN, pars nervosa; PT, pars tuberalis.

would seem to confirm de Beer's statements. The purpose of the present study was to determine the vascularity in the lobes of a turtle, an animal which is believed to be related to the stock from which mammals arose.

Materials and Methods

The first seven of the specimens had been injected, sectioned, and mounted for Dr. E. Horne Craigie for his studies of the vascularity of the reptilian brain (1941 (12)). The author is extremely grateful for access to this material. The method used for the remaining three specimens was essentially the same as that described in the work cited above in which carmine gelatin was injected, and the material was fixed in 10% formalin and sectioned 20μ thick, with the exception of each 11th section which was 100μ thick in order to provide a slight check on the completeness of the injection.

As in the previous work of this kind (Taylor and Craigie, 1938 (38)), measurements were made on 10 successive sections and the length of capillaries was measured in a constant volume of tissue, which, in this case was a rectangular block of tissue 242μ square and 200μ thick. The measured length of vessels obtained was corrected for the length that they would have in 1 cu. mm. of fresh tissue. The percentage loss for shrinkage, 14.8%, and the correcting factor for shrinkage, 0.71 (Craigie, 1924 (8)), were those calculated for the turtle brain (Craigie, 1941 (12)). There is no certainty that the correction for the shrinkage of the hypophysis would be the same as that for the brain. However, this might not introduce a large source of error as Stevens (1937 (37)) obtained for shrinkage of the hypophysis of the cat a value which was very close to that obtained for the brain of the rat (Craigie, 1924 (8)).

The possibility of active contraction of the capillaries in the conditions of this experiment is discussed by Stevens (1937 (37)). She concluded that the only way to ensure complete injection is to use high pressures. The pressures

used, values up to 300 mm. of mercury, while the normal blood pressure of the turtle is 23 to 50 mm., should have been sufficient. It might be suspected that the diameter obtained might be affected by the pressures used. However, as there was no correlation between diameter of vessels in any lobe and the pressure used, it is apparently not a large source of error.

Results

Length and Diameter of Vessels

Table I shows the lengths of capillaries in the 1 cu. mm. volume of fixed tissue. Here also are shown the lengths in the same volume of tissue corrected for shrinkage. It is seen that the most vascular tissue is the pars tuberalis

TABLE I

TOTAL LENGTH OF CAPILLARIES IN 1 CU. MM. OF TISSUE OF HYPOPHYSIS OF PAINTED TURTLE

	Animal No.										Average	C of V	Length corrected for shrinkage in 1 cu. mm. of fresh tissue
	♀33	♀47	♀48	♀49	♂50	♂51	♂54	♀201	♀215	♀217			
	Length, mm.												
Pars nervosa	427	242	397	348	381	409	522	381	345	345	379	6.0	269 ± 16
Pars intermedia	376	288	328	237	331	444	350	324	292	476	345	6.7	245 ± 16
Pars distalis or pars anterior	922	535	667	692	615	742	575	724	802	691	697	5.1	495 ± 25
Pars tuberalis	1570	780	833	1160	1010	879	801	1210	965	1090	1030	7.5	730 ± 55

followed in order by the pars anterior, the pars nervosa, and the pars intermedia. Compared with the vascularity of the latter, their relative richness is in the following ratios:

$$\frac{\text{pars anterior}}{2.0} = \frac{\text{pars intermedia}}{1.0} = \frac{\text{pars nervosa}}{1.1} = \frac{\text{pars tuberalis}}{3.0}.$$

This variation in capillary richness is also apparent from the photomicrographs (Figs. 2 and 3). In Fig. 3 the blood vessels of the pars nervosa are shown to be very close to the adjacent part of the intermediate lobe, so that they must supply it also. The lengths of such vessels were consequently taken as belonging one-half to either lobe. The difference between the measurements for these two parts is probably not mathematically significant, 401 ± 340 mm., but differences between any other two lobes are of definite statistical validity (greater than twice the standard deviation of their mean differences).

A comparison with the capillary lengths in corresponding lobes of the frog (Taylor and Craigie, 1938 (38)) and of the cat (Stevens, 1937 (37)) is shown in Table II. In the frog, the pars nervosa has the greatest length, followed by the pars anterior, pars tuberalis, and pars intermedia. In the cat, the order

PLATE I

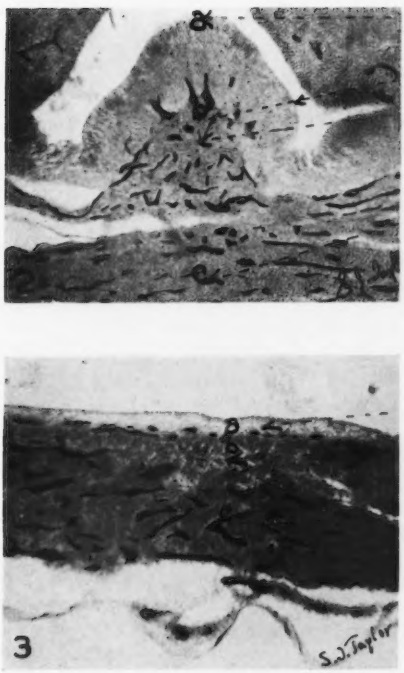

FIG. 2. Photomicrograph of transverse section 20μ thick hypophysis of *Chrysemys picta marginata* × 100; *a*, floor of third ventricle; *b*, pars tuberalis; *c*, pars anterior.

FIG. 3. Photomicrograph of transverse section 20μ thick of *Chrysemys picta marginata* × 100; *a*, pars nervosa; *b*, pars intermedia; *c*, pars anterior.

TABLE II[2]

VASCULARITY OF HYPOPHYSIS IN 1 CU. MM. OF FRESH TISSUE

	Length of capillaries, mm.				Diameter of capillaries, μ			
	Painted turtle	Leopard frog	Salamander	Cat	Painted turtle	Leopard frog	Salamander	Cat
Brain (average)					7.1	5.6		3.2 (Rat)
Pars nervosa	269	634		698	8.0	10.1		3.8
Pars intermedia	245	97	74.1	70	8.1	10.5	11.3	3.8
Pars anterior	495	510	389	936	11.1	8.8	8.3	7.7
Pars tuberalis	730	256		789	7.6	9.2		8.6

[2] *Data from the following sources: leopard frog—Taylor and Craigie (1938 (38)); salamander—calculated from Craigie (1938 (11)); cat—Stevens (1937 (37)); turtle brain—Craigie (1941 (12)); rat brain—Craigie (1920 (7)), (1931 (9)).*

of decreasing capillary length is pars anterior, pars tuberalis, pars nervosa, and pars intermedia. From the same table, if one compares the capillary richness for each part for the animals studied, it appears that both for the pars anterior and for the pars nervosa, the richness decreases in the order of cat, frog, and turtle. The pars intermedia, however, is least vascular in the cat and most vascular in the turtle. In the pars tuberalis the highest value is obtained from the cat and the lowest from the frog.

The average diameter of capillaries in the lobes of the turtle's hypophysis appears in Table II, as well as a comparison with the corresponding lobes in the cat, frog, and salamander.

The tables and photomicrographs indicate that in the turtle the vessels of the anterior lobe are larger than in the other lobes and in the surrounding brain tissue. The differences in diameter of the capillaries in other lobes from one another and from those in brain tissue are not of mathematical significance. This greater diameter of the vessels in the anterior lobe is in contrast to the conditions in the frog. In the latter animal the capillaries are wider in all lobes than in the brain and are narrowest in the anterior lobe. The findings for the turtle are similar to those for the cat in that the vessels of the anterior lobe are larger than in neural and intermediate lobes and in brain tissue, but differ in that, in the pars tuberalis of the turtle, the average diameter of the capillaries of the pars tuberalis is wider than those of any of the other lobes or of the brain.

Volumes of Capillaries and Areas of Their Walls

Since the vascularity of a tissue depends on the diameter of the vessels as well as on their length, the capillary richness can be shown by the volume of capillaries in a unit volume of tissue better than by length alone. The volume of the capillary network, the area of its walls, and a comparison with the cat (Stevens, 1937 (37)), frog (Taylor and Craigie, 1938 (38)), and salamander (Craigie, 1938 (11)) are shown in Table III and in Fig. 4.

TABLE III[3]

VOLUMES OF CAPILLARIES AND AREA OF THEIR WALLS IN THE HYPOPHYSIS OF THE PAINTED TURTLE COMPARED WITH LEOPARD FROG, CAT, AND SALAMANDER IN 1 CU. MM. OF FRESH TISSUE

	Painted turtle		Leopard frog		Cat		Salamander	
	Volume of capillaries, cu. mm.	Surface area, sq. mm.	Volume of capillaries, cu. mm.	Surface area, sq. mm.	Volume of capillaries, cu. mm.	Surface area, sq. mm.	Volume of capillaries, cu. mm.	Surface area, sq. mm.
Medial longitudinal fasciculus	3.60×10^{-3}	1.92	3.30×10^{-3}	1.70	(Rat) 3.10×10^{-3}	(Rat) 3.50		
Cochlear nucleus	13.8×10^{-3}	7.80	14.3×10^{-3}	8.60	(Rat) 10.60×10^{-3}	(Rat) 12.2		
Pars nervosa	13.3×10^{-3}	6.64	51.84×10^{-3}	20.1	7.91×10^{-3}	8.33		
Pars intermedia	12.6×10^{-3}	6.03	8.61×10^{-3}	3.25	$.79 \times 10^{-3}$	.835	7.42×10^{-3}	2.63
Pars anterior	48.0×10^{-3}	17.20	31.0×10^{-3}	14.75	43.6×10^{-3}	22.6	21.05×10^{-3}	10.15
Pars tuberalis	37.8×10^{-3}	18.30	17.01×10^{-3}	7.40	45.8×10^{-3}	21.3		

[3] *Data from the following sources: frog brain and hypophysis—Taylor and Craigie (1938 (38)); turtle brain—Craigie (1941 (12)); cat hypophysis—Stevens (1937 (37)); salamander hypophysis—Craigie (1938 (11)); rat brain—Craigie (1920 (7)) and (1931 (9)).*

Table III shows that in the turtle the capillary richness as measured by the surface area in a unit volume of tissue is of the same order of magnitude in pars intermedia as in pars nervosa. The surface of the capillaries in partes anterior and tuberalis is between two and three times as great as in the former lobes. If one considers volume of blood vessels in a unit volume of tissue as an index of capillary richness, the vascularity of the pars anterior is seen to be still greater, between three and four times that of the intermediate and neural lobes.

Comparing the blood supply of the turtle's hypophysis with that of the brain, it is apparent from Fig. 4 that not only is the extent of the capillary

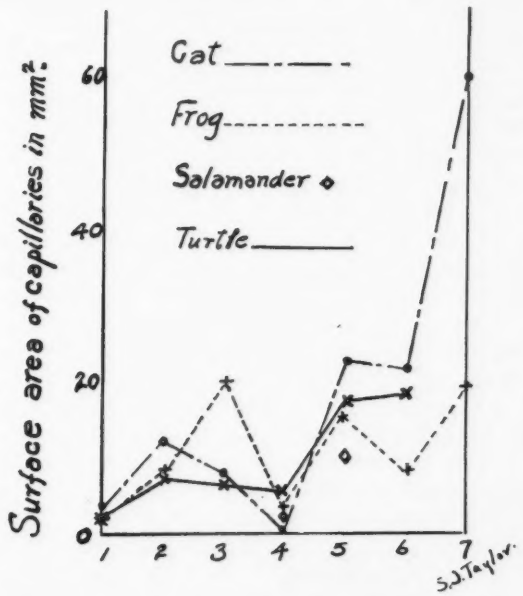

FIG. 4. Graph showing the surfaces of capillaries in 1 cu. mm. of fresh tissue in turtle hypophysis compared with brain and muscle of frog, salamander, turtle, and cat. 1. Medial longitudinal fasciculus. 2. Cochlear nucleus. 3. Pars nervosa. 4. Pars intermedia. 5. Pars anterior. 6. Pars tuberalis. 7. Skeletal muscle.

bed in even the poorest lobes of the hypophysis much greater than in the poorest parts of the brain, the medial longitudinal fasciculus, but in these lobes, intermediate and neutral, it approximates the vascularity of the richest centers of the brain stem, the cochlear nucleus. A comparison between the extent of the capillary system in hypophysis and in muscle of the turtle would also be of interest, but the latter data do not seem to be available.

A comparison of the surface areas of the vessels of the different lobes of the turtle hypophysis with those in the frog, salamander, and cat can be made from Table III and Fig. 4. From Fig. 4 it appears that the areas in the

turtle are lower than, but parallel to, those in the cat with, however, some exceptions. In the turtle there is less variation from lobe to lobe and the intermediate lobe in the turtle is more vascular than in the cat. This graph shows, too, a contrast between the turtle and the frog. In the latter animal the neural lobe is much more vascular and the tuberal and anterior lobes are much less vascular than in the turtle or in the cat. In the salamander the pars anterior and pars intermedia are less vascular than in the turtle.

Discussion

Most evidence indicates that, with the exception of muscle, the vascularity of a tissue is related to the functional requirements of that tissue. Against this premise is the work of Gerard (1938 (16)) which showed that the ratio of vascularity of cerebral cortex to peripheral nerve was much less than the ratio of their metabolic requirements. However, Scharrer (1939 (36)) found that in the cerebellum of the monkey the capillaries were placed at such a distance that metabolic requirements would be met. Craigie (1924 (8)) recorded that as young rats developed the capillary richness of the brain increased. Petren's experiments (1938 (28)) showed that alterations of capillary extent may occur in response to functional needs—that motor but not sensory centers became more vascular after enforced exercise. Lindgren (1940 (23)) observed in the human brain a reduced vascularity with diminution of tissue.

If the vascularity of a tissue is related to its functional significance and if the functional significance changes with position on the evolutionary scale, then variations in vascularity of a certain part would show a phylogenetic relation. Such a relation was noted by Craigie (1938 (10)) from his studies of the vascularity of the nervous system: "There is a trend, though an irregular one, in the direction of vascular richness and greater differentiation in respect to vascularity in specific centres of animals with higher rank in the evolutionary scale". From Fig. 4 it is noted that the partes anterior and tuberalis increase in vascularity as the evolutionary scale is ascended, while the partes nervosa and intermedia decrease in the same order, except that in the turtle the pars nervosa is the poorest and the pars intermedia is the richest. Many more representative species would be necessary to fill in the details of this possibility.

One other factor which should be considered is the relation of metabolic rate to the size of the animal. The larger the animal the smaller is its metabolic rate and the fewer are the blood vessels needed to meet tissue requirements. From studies on the vascularity of the nervous system Craigie (1938 (10)) noted that there is a progressive decrease in vascularity of a certain region of the brain with the size of the animal. If the factor of size were the only one involved, the vascularity of the pars anterior and pars tuberalis would be in the reverse order. However, according to Benedict (1932 (3)) the turtle has a higher general metabolic rate than most cold blooded animals but lower than the warm blooded ones. This would act in the opposite fashion to the size of the animal and would not account for the fact that one of the

least vascular tissues is met in the cat. In Fig. 4 it appears, however, that the graph line for the turtle is lower than, but follows that, of the cat. This suggests that the general metabolic rate of the different animals may be one contributing factor.

In an attempt to determine the significance of the vascularity observed, it is necessary to consider the functions of the pituitary and to see how the importance of the lobes differs among the classes of animals studied. The general picture is an increase in importance of the pars anterior, a decrease in the pars intermedia, and questionable variation in the partes tuberalis and nervosa.

At least five hormones have been reported in the anterior lobe of the pituitary in reptiles (Houssay, 1930 (18), Evans, 1936 (14), Forbes, 1937 (15), Risley, 1939 (33), Neeser, 1940 (25), Cunningham and Smart, 1934 (13), Riddle *et al.* 1935 (32), Leblond and Noble, 1937 (22), Meyer, Mellish, and Kufferman, 1939 (24), Schaefer, 1933 (35), Roth, 1942 (34), Houssay and Biasotti, 1933 (19)). If prolactin- and lutein-stimulating hormones which occur in mammals, are represented in the turtle, they would appear to lack "target organs" so that the anterior lobe might be expected to be less active and hence less vascular.

The intermediate lobe of amphibians and some reptiles is responsible for melanophore-contraction with dispersion of melanin and resultant darkening of the animal (Noble and Bradley, 1933 (26), Kleinholtz, 1938 (20), Parker, 1938 (27), Rahn, 1941 (31)). This effect is of no apparent significance in the turtle or in mammals. The greater vascular richness of the pars intermedia in the frog may be related to this but its richness in the turtle which has no actively contractile pigment cells is not thus applicable.

The special action of the pars tuberalis of reptiles does not seem to have been investigated.

For certain reptiles extracts of the pars nervosa have an oxytocic (Clausen, 1940 (6)) and a vasopressor action (Burgess, Harvey, and Marshall, 1933 (5), Van Dyke, 1936 (39), 1939 (40), Boyd and Dingwall, 1939 (4)). However the differences in vascularity in the animals under consideration cannot be consistently correlated with the production of these substances.

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THE EFFECTS OF RADIATION ON THE HABITAT TEMPERATURES OF THE LODGEPOLE NEEDLE MINER, *RECURVARIA MILLERI* BUSCK (GELECHIIDAE : LEPIDOPTERA)[1]

By W. R. Henson[2] and R. F. Shepherd[3]

Abstract

The lodgepole needle miner (*Recurvaria milleri* Busck) passes the greater part of its life in the interior of needles of its host tree. Investigations of the temperature of needle mines and its relation to ambient air temperature, radiation, and a number of other weather factors are reported. Incoming solar radiation shows a heating effect on the needle mines which is directly proportional to the radiation level. The heating effect of the radiation is modified by other weather factors, by differences in the exposure of the needles, and by the type of mines in the needles. At night, needles are cooled below ambient air temperatures by outgoing radiation, which in turn is dependent on the nocturnal weather. The application of these results to studies of the effect of temperature on the needle miner can only be made with respect to individual needles. Thus, radiation of 1.5 gm-cal. per cm.^{2} per min. will elevate the temperature of a needle 6.3 Centigrade degrees in air moving at less than one mile per hour if the needle is orientated at right angles to the sun and fully exposed. Shade from other needles, wind over one mile per hour, and needle orientation other than 90° to the sun's rays all tend to reduce the amount of heating at the indicated level of radiation.

Introduction

During 1950, a program of bioclimatological research was started in the national parks area of the Rocky Mountains in Alberta and British Columbia. The program, a part of the work of the Section of Bioclimatology of the Division of Forest Biology, was organized to investigate problems relating to the forest insect pests of the area. This paper deals with an investigation of the temperatures of the habitats of the lodgepole needle miner.

The temperature of insectan habitats is seldom that of the ambient air (3). This fact has been recognized by a number of workers in the past but, despite this recognition, many attempts have been made to investigate the effects of temperature on insects in the field making use of air temperature data. The fact that the temperatures of plant tissues rise well above those of the surrounding air under the influence of solar radiation is well known (1). That these tissues fall below air temperature by radiational cooling is not so widely recognized. The modification of the temperature of plant tissues by both incoming and outgoing radiation will, however, affect the body temperature of any insect which resides within or on the tissues. Such temperature modifications are of great importance in any attempt to investigate the temperature regime under which an insect is living. The radiational heating and cooling effects which apply to the tissues of needles, also apply to some extent directly

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to insects within the plant tissues. Thus, the temperature of an insect body may differ from that of surrounding plant tissues while the plant tissues differ in temperature from the surrounding air.

The lodgepole needle miner is of considerable importance in the stands of lodgepole pine which cover a great deal of the lower parts of the Rocky Mountain National Parks of Canada. The insect has been known in the Rocky Mountains for some time and has been the subject of a number of investigations by members of the Calgary and Vernon Forest Insect Laboratories (2). The larval and pupal stages are passed in the interior of the needles of the host tree. Eggs are deposited on the surface of the needle and the emergent larva enters the needle near the distal end. The period of development is two years in the area under discussion. Larval feeding takes place throughout the entire season when temperatures are favorable. Numerous observations indicate the importance of temperature to the survival and growth of the insect. The present work is an attempt to provide a basis on which temperature regimes may be evaluated.

Materials and Method

The measurements were taken on the north-facing slope of Cathedral Mountain in the Kickinghorse Canyon opposite the south opening of the Yoho Valley in Yoho National Park, B.C. The stand on this slope is even-aged fully stocked lodgepole pine ranging between 15 and 40 ft. in height.

In most cases, there is no overlapping of the cycles of development of this insect so that adults are only found during alternate years. However, the needle miner infestation in the area during 1951 was unusual in that larvae were found in two separate stages of development. This fortunate anomaly in the population provided a source of needle mines in all stages of development.

The mines vary in size and type as the feeding of the insect progresses. In general, mines may be divided into two kinds according to their stage of growth. The first kind of mine is one which does not occupy the whole of the cross section of the needle. Such mines show as a light, sometimes tortuous streak leading down the needle from an entrance hole near the tip. Some of the mesophyll of the leaf remains and the tissues of the needle do not commonly die, so that the whole of the needle retains its green color. The second class of mine is one in which all the mesophyll of the cross section of the needle is removed. Here, only the epidermis is left and rapidly turns brown.

The measurements of the needle mine temperatures were accomplished by the use of copper–constantan thermocouples. The couples, made from 30 gauge duplex glass insulated wire, were inserted into the needle mines from the cut distal ends. The short leads were clamped to the twig so that the thermocouple approximated the position of a feeding larva in the mine. The standard type of mine used was one in which all the mesophyll of the needle had been removed during the current year.

A portable potentiometer was used for measurements with the thermocouples. The instrument was calibrated to read directly in degrees Centigrade

with the copper–constantan junctions. It was fitted with long leads of duplex wire which permitted connection to each of a series of thermocouples in turn. The thermocouples were left in place throughout the whole of the observations, a period of about a month during July and August.

Ambient air conditions were measured by means of a sling psychrometer or unshielded wet and dry thermocouples mounted in the shade of the tree under observation. Radiation was measured in terms of gram-calories with a small portable meter. Clouds and other weather factors were recorded at the time of each observation.

The procedure for an observation consisted of taking a reading of the ambient conditions, a reading of the radiation level in the open, measurements of the temperatures of the needles, and finally a confirmatory reading of the air temperature and radiation level. Observations made during times of rapidly changing conditions had to be discarded. The amount of change in the ambient conditions which was tolerated during a five minute period of observation was roughly 0.5°C. temperature change and a radiation change of 0.1 gm-cal. per cm.^{2} per min.

Results

At each level of radiation, the mine temperatures varied according to the exposure and the orientation of the needles to the source of radiation. Table I shows the temperature of one needle mine when orientated in different ways

TABLE I
THE INFLUENCE OF NEEDLE ORIENTATION ON THE AMOUNT OF RADIANT HEATING OF MINES AT CONSTANT HIGH INSOLATION

Needle orientation															
0°			45°			90°			135°			180°			
T	*B*	*S*	*T*	*B*	*S*	*T*	*B*	*S*	*T*	*B*	*S*	*T*	*B*	*S*	
3.8	4.5	4.9	6.1	6.5	6.1	7.7	7.9	7.9	5.1	4.2	4.2	2.0	1.5	1.5	

to the sun's rays. The side of the needle which is exposed, as well as the angle of the needle as a whole, affects the amount of heating. In the table, the convex top (*T*), the flat bottom (*B*), and the sharp side (*S*) are distinguished. The angle of orientation is the angle subtended by the long axis of the needle from the direction of the sun's rays. At 180°, the distal end of the needle was thus pointing directly at the sun. At this angle, the needle was partly shaded by the lead wire. When the needle was pointed directly at or directly away from the sun, differences due to position of the needle were still recorded. This may be traced to the high level of radiation from the sky. Air temperature during the readings reported in Table I varied between 25.3°C. and 24.6°C. Radiation was constant at 1.40 gm-cal. per cm.^{2} per min. Readings are given as Centigrade degrees above air temperature.

Small inconsistencies in this table are the result of a lack of accuracy in the orientation of the needle. The orientation of the needle is of considerable importance in changing the amount of heating which may be produced by a given level of radiation. Smaller differences may be assumed under less intense radiation.

Another source of variation in the heating effect of the sun on the needle mines lies in the nature of the mine. Here, two factors seem to be important. The first is the color of the mine. The second is the size of the cross section of the mine, that is, the amount of the mesophyll removed. These two factors vary together. As feeding progresses, the insect removes more and more of the contents of the needle and the remaining tissues darken. Table II

TABLE II

THE RELATIONSHIP BETWEEN MINE SIZE AND HEATING AT CONSTANT RADIATION

	Position of needle on tree			
	North	East	South	West
Green needle	1.4	2.3	3.2	3.3
Small mine	1.3	1.2	2.2	2.0
Large mine	1.3	2.0	5.4	3.5

presents a representative set of readings which show the relationship between the amount of heating produced by a constant level of radiation on large and small mines and on green needles. Needles at the top of the twig were used for these measurements in order to obtain a constant angle of orientation. The effect of needle position on the tree is also shown on this table. The air temperature during the readings was constant at 25.4°C. The radiation was constant at 1.49 gm-cal. per cm.^{2} per min. The sun was in the southwest at the time of the readings.

Small mines are heated the least at the level of radiation observed. The heating of green needles is intermediate, and of large mines, greatest. These differences are due to the gradation in color between the three structures. The large air space in the large mines probably accounts for some of the heating because in the region of the needle which is mined, normal transpiration is halted and an important cooling mechanism removed. The effect of mutual shading of the various parts of the tree is of the same order in all three types of needles; this is shown by the relationship between the readings from the four quadrants.

Wind has a very pronounced effect on the temperature of the needle mine. Air movement always resulted in the approximation of the temperature of the mines to that of the surrounding air. At velocities of one to two miles per hour some effect could be detected. At velocities greater than 7 to 10 miles per hour, the temperature of the needle mines was that of the ambient air.

Wellington (4) found that air movement did not have much effect on the temperature of the interior of insect galleries formed of silk-webbed balsam fir foliage. The smaller size of the mines in lodgepole pine needles probably accounts for the difference.

Table III presents a summary of the relationship between the radiation level and the amount of heating of needle mines. The readings were taken in still air when radiation levels were constant. Readings from five fully exposed needles were used. At least five series of readings were taken at intervals of radiation level of approximately 0.1. A curve was fitted to these data and readings taken off the curve at regular intervals. The readings in Table III

TABLE III

THE RELATIONSHIP BETWEEN RADIATION LEVEL AND THE AMOUNT OF HEATING OF NEEDLE MINES

Radiation level	0.0	0.1	0.2	0.3	0.4
Mine temp.—air temp.	−0.8	−0.3	0.1	0.6	1.1
Radiation level	0.5	0.6	0.7	0.8	0.9
Mine temp.—air temp.	1.6	2.0	2.5	3.0	3.5
Radiation level	1.0	1.1	1.2	1.3	1.4
Mine temp.—air temp.	3.9	4.4	4.9	5.3	5.8
Radiation level	1.5	1.6			
Mine temp.—air temp.	6.3	6.8			

are not as high as those reported in Table I at maximum exposure. This is because the readings were taken on needles *in situ* which were not orientated at exactly 90° to the sun's rays. The radiation levels in the table are given in gm-cal. per cm.^{2} per min. The differences of mine temperature from air temperature are given in Centigrade degrees.

The elevation of the temperature of the needle mines is directly dependent on the level of radiation, and, at temperatures in the range encountered during summer days in the Rocky Mountains (6° to 30°C.), it is relatively independent of air temperature. That the mine temperatures will be modified by wind has already been noted. Small amounts of shade will reduce the heating effect in mines; the shade from only one needle is sufficient to reduce the temperature of a mine markedly.

When the radiation level is changing rapidly, the temperature of the needle mine lags behind the changing radiation level. When radiation is increasing, the mine temperature takes about a minute to reach stability at a new radiation value if the change is small (less than 0.2 gm-cal. per cm.^{2} per min.). Larger changes in radiation level result in longer lags. When the radiation is decreasing, the temperature of the mine falls rapidly and frequently "overshoots". For example, when the radiation level fell from 1.4 to 0.4 gm-cal. per cm.^{2} per min. as a cloud passed over the sun, the temperature elevation in a medium sized mine fell from 5.8° to almost 0.0° and then, within a few

minutes, rose again to 1.1°. This phenomenon was due to rapid radiational cooling and to the persistence of a high transpiration rate which cooled the needle beyond the point consistent with the new radiation level. Overshooting is most clearly seen at the beginning of the night period when the radiation value is so low that the cooled needles are not brought back to a level consistent with the radiation level for some time.

Radiation level varies in different parts of the crown of a tree. The most important source of this variation is to be found in the mutual shading of the branches. Table IV gives values of radiation taken from four positions

TABLE IV

THE EFFECT OF POSITION IN THE TREE ON THE AMOUNT OF RADIATION FALLING ON NEEDLES

Time	Sun bearing	Radiation in open	North		East		South		West	
			B	*T*	*B*	*T*	*B*	*T*	*B*	*T*
1110	174°	1.45	0.00	0.26	0.55	1.35	1.05	1.35	0.45	1.32
1325	210°	0.80	0.10	0.35	0.35	0.45	0.50	0.50	0.35	0.75
1345	215°	0.40	0.05	0.20	0.25	0.40	0.30	0.35	0.15	0.40

around an exposed tree at three radiation levels. These values cannot be closely compared because of changes in the elevation and compass bearing of the sun. The values are only indicative of the order of differences to be expected. The readings were obtained with the meter held in the foliage in the position indicated. In each quadrant, readings were taken among the needles on the top (*T*) and bottom (*B*) of the twig. Maximum readings from each position were used to approximate the maximum radiation level to which a needle in that position could be subjected. Inconsistencies in the table are due to the fact that the crown of the tree was not of uniform density and the amount of mutual shading between the various parts of the tree was not constant. The radiation readings in the table are given in gm-cal. per cm.^{2} per min.

The temperature of the needle mines at the various points around the tree should be consistent with the radiation levels at those points. Radiation from the sun and sky is, of course, the main source of the heat which elevates the mine temperatures. However, there is a certain amount of reradiation from the ground. This results in slightly higher temperatures in the mines on the bottom of the twigs than would be expected from the readings of solar radiation. The effects of ground radiation are most clearly evident during the early part of the night when the ground is warmer than the air.

On the basis of the data given previously, an estimate may be made of the temperature of any given mine. For example, a mine on the top of a twig on the north side of the tree, when the sun is at 180° and the radiation at 0.80 gm-cal. per cm.^{2} per min. will receive 0.35 gm-cal. per cm.^{2} per min. of radiation. In still air, the temperature of the mine would be about 0.7°C. above

the ambient temperature. At the same radiation value a needle on the top of a twig on the south side of the tree will receive between 0.50 and 0.75 gm-cal. per cm.^{2} per min. and in still air, the temperature of the mine will be between 1.6 and 2.5°C. above the ambient temperature. Both these cases have assumed that the needle was fully exposed. Shade on the needle or a needle orientation other than 90° to the sun's rays, would reduce the amount of heating.

Estimates may thus be made by determining the position of the needle on the tree and on the branch with respect to the sun, determining the ambient radiation value and making an estimate of the radiation received at the needle (Table IV). A temperature reading is then taken from Table III and corrected for orientation from Table I and for mine type from Table II. These estimates are limited to relatively still air and dry foliage.

Fig. 1 gives a scatter diagram of the relationship between estimates made by this method from field data and the actual readings as determined with thermocouples. Absolute accuracy in the estimates would give a line of points in the scatter diagram with slope 1.0 passing through the origin. The small estimates at the bottom of the scale probably are the result of the overshoot effect which was not considered in the correction.

The course of the temperature curve inside needle mines during the night is of considerable interest. The radiation values at night are so low that it has not been possible to obtain measurements. Reradiation from the ground is in the form of long-wave radiation which is not detected by the instrument used. Thus, statements about the radiational heating and cooling of the needle mines at night must be, to some extent, conjectural.

As the radiation decreases through the early evening, the temperature of the needle mines follows the decrease as outlined in Table III. Just after the sun has set, the needle mine temperatures drop rapidly until they are about one Centigrade degree below air temperature. This condition persists for about fifteen minutes, when the temperature of the mines returns approximately to air temperature. On clear nights, the temperature of the mines follows that of the air very closely with mined needles on the top of the twigs about half a Centigrade degree colder than the air and mines on the bottoms of the twigs about a quarter of a degree colder than the air. The air temperature range on clear nights in the Kickinghorse Valley was between 6° and 11°C. on all clear nights during which observations were made. At higher temperatures somewhat greater differences between mine temperature and air temperature could be expected owing to the higher radiation rates (4). The lower temperature of the mines on the tops of the twigs is partly due to a greater exposure of the top mines which permits free radiation to the sky. Reradiation from the ground contributes to the difference by warming the bottom mines more than those on the twig tops.

On clouded nights, the temperature of the needle mines follows a course similar to that described for clear nights through the period of overshooting. The needle mines become considerably colder than the air and then, after the

overshoot period, return to air temperature. As the air temperature drops during the night, the needle mine temperatures decrease at a lower rate so that the mine temperatures on cloudy nights are slightly higher than the air temperatures.

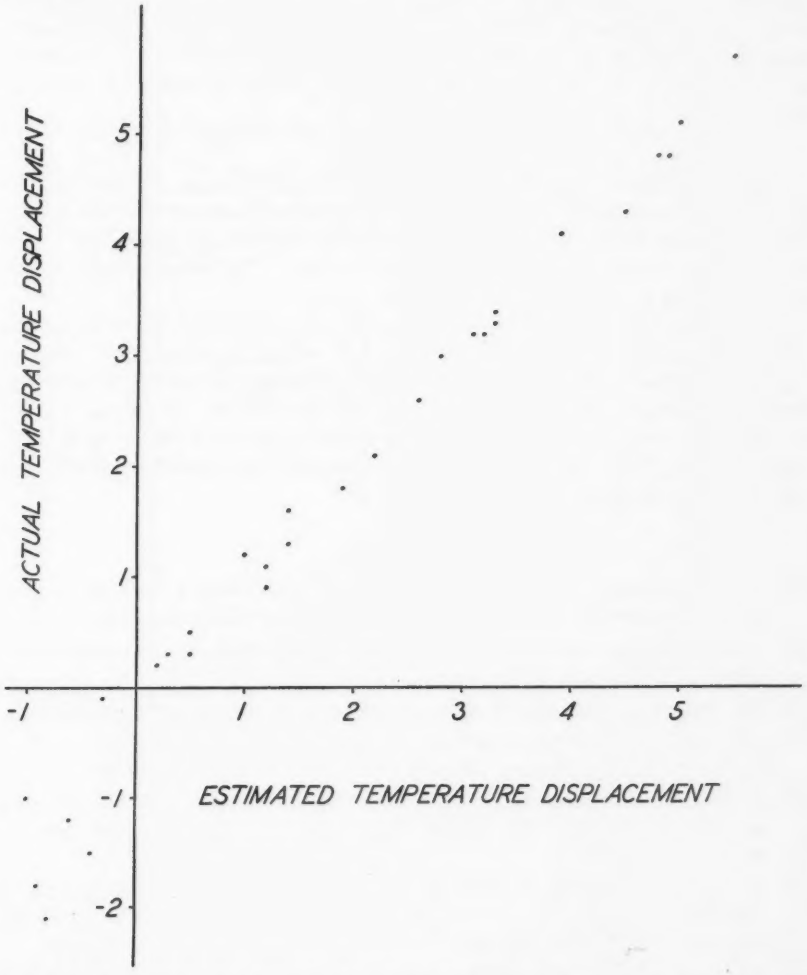

FIG. 1. The relationship between the actual temperature displacement and estimated temperature displacements in mines of the lodgepole needle miner.

During both night and day, the presence of free water on the foliage cools the needle mines. This effect is due to evaporational cooling and the removal of heat from the needles by conduction. The deposition of dew on the foliage is accompanied by a slight rise followed by a sharp drop in the temperature

of the needle mines. The initial rise is evidence of the heat liberation which takes place when the air reaches its dew point and the heat of vaporization of the atmospheric water is set free.

Wind causes a breakdown of all the temperature differences which have been described for the night period. The velocity of wind which causes this breakdown is even smaller than that required during the day. Wind of three to five miles per hour warms all needle tissues to air temperature except when there is free water on the foliage, in which case, the foliage is cooled to wet bulb temperature.

Discussion

The observations reported here should make possible more accurate studies of the relationships between the temperature and the growth of the needle miner. From these findings, it is possible to make close estimates of the temperature inside any individual mine at any time when foliage is not covered by snow.

A great deal of mortality has been observed among needle miner populations during the winter. The studies reported here should be extended to include the winter period. Although some work (4) has been reported on the temperature conditions inside coniferous foliage during the winter, so far as the authors are aware, such measurements have never been made on a two-needled pine. The high exposure of the foliage and the unique weather of the mountain areas make such work desirable.

Conclusions

1. The temperature regime of an insect living within the foliage of its host cannot be assumed to be equivalent to the regime of air temperature.
2. An investigation was made of the effects of radiation on the temperature of the mine habitats of the lodgepole needle miner.
3. The orientation and aspect of the needle, the degree of development of the mine, and the presence or absence of wind, as well as a number of other factors modify the heating effect of radiation on the needle mines.
4. A curve of the relationship between radiation and the amount of mine heating was derived. It showed that the relationship is linear and extremely regular.
5. At any constant level of radiation, a regular distribution of radiation levels around a tree is found. The mutual shading of the various parts of the trees accounts for the different levels of radiation at various positions.
6. Needle mine temperatures at various points around the tree were predicted from field data and the results shown to correspond to the results of direct measurements.
7. The course of the needle mine temperatures throughout the night was followed. On clear nights the mine temperatures are below air temperature, while on clouded nights, mine temperatures are slightly higher than air temperature.

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A STUDY OF SIZE INHERITANCE IN THE HOUSE MOUSE

II. ANALYSIS OF FIVE PRELIMINARY CROSSES[1]

BY L. BUTLER[2]

Abstract

Five crosses were made between mice of different body size, and over 2000 mice were raised in the F_1, F_2, and backcross generations. The body weight at 60 days after birth was used as the criterion of size. The male means were always larger than the corresponding female means and the difference in weight between the two sexes increased progressively with body size. Litter size and sequence had no effect on body size. The adequacy of the gram scale was tested with inconclusive results which indicated that in at least two crosses some other scale should be used. Log-grams were substituted for grams and gave a good fit in cross No. 3 but not in crosses Nos. 1 and 2. The evidence from selection experiments, environmental variability, and sex differences in size indicate that on a gram scale at least part of the factors which affect body size are proportionate rather than additive in nature. In all five crosses the F_1 and F_2 means are intermediate between the parents. The backcross means are halfway between the F_1 and the respective parent. Only one cross showed increased size in the F_1 which might be interpreted as due to heterosis. Reciprocal crosses gave significantly different results and the dissimilarity was carried over into the next generation. This difference was attributed to the environmental effects of female body size. As expected, the variances of the P_1's and the F_1 were similar but, contrary to expectation, the F_2 variance was no larger than that of the F_1. Litter size showed a different type of inheritance. One cross between P_1's with mean litter sizes of 5.1 and 10.2 gave an F_1 mean of 13.2 young. This was tentatively interpreted as dominance of large litter size and hybrid vigor allowing more embryoes to reach parturition.

Introduction

The inheritance of quantitative characters such as body size is of great importance both from the scientific and practical standpoint. The mouse furnishes excellent material for this purpose since it can be cheaply and quickly raised in fairly large numbers. There are strains which run from 10 to 50 gm. in body size, will cross freely, and will produce large litters. Before efficient experiments can be planned, it is necessary to know what crosses to make, how many animals to raise, and how to handle the rather complex data. In order to find answers to as many of these questions as possible the five crosses reported here were made.

Parental Stocks

The parental stocks consisted of MacArthur's large and small strains, and two inbred stocks of normal size with genotypes *DBa'* and *pbs*. MacArthur's strains were not inbred; they were developed from heterogeneous stock of normal size by mass progeny selection (MacArthur (4)). It was expected that the differences in body size between the parent strains were great enough to make up for their lack of genetic purity. In fact the decrease in body size

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which generally accompanies inbreeding may introduce more error into the experiments than the lack of homogeneity.

After the stocks were obtained from MacArthur, his system of selection and the tabulation by generations was continued with some slight modifications. The number of mice raised in each generation, together with their average weights and litter sizes, is given in Table I. The data indicate that

TABLE I

WEIGHT AND LITTER SIZE DATA FOR THE PARENT STOCKS

	Males			Females			Litter size	
	No.	Mean	Variance	No.	Mean	Variance	Birth	60 days
L strain								
Generation 21	117	39.85	25.85	109	34.46	26.17	10.1	8.7
" 28	100	42.07	30.87	101	36.04	29.29	9.0	6.9
" 29	165	39.62	24.20	161	32.01	23.10	9.7	8.0
" 30	79	33.61	16.16	77	29.05	7.84	10.2	8.8
" 31	101	31.44	21.86	86	27.44	12.52	9.3	7.2
" 32	108	33.73	9.79	76	30.35	5.85	9.0	7.4
" 33	108	29.90	14.28	123	26.53	10.48	10.0	8.5
Three litter set	64	30.88	12.06	62	28.65	18.94	11.1	8.4
Four litter set	158	30.27	14.91	134	27.56	17.75	11.3	9.1
S strain								
Generation 21	36	11.97	2.82	33	10.79	2.16	5.1	3.6
" 28	72	13.27	6.07	62	11.32	5.86	5.8	4.5
" 29	27	13.74	8.64	19	12.33	2.52	5.1	4.0
" 30	48	13.07	2.09	60	10.81	2.72	5.5	4.3
" 31	77	13.24	8.21	68	11.77	2.59	5.5	4.0
DBa^4 stock	95	22.42	8.51	86	18.85	9.12	6.8	6.0
pbs stock	74	21.34	7.92	62	18.64	8.81	7.1	6.0
pbs stock	51	21.71	6.21	43	20.18	3.11	7.3	6.7

selection after the 21st generation was not effective. In fact measures of heritability have negative values because in many cases the mean of a generation differs from the preceding generation in a manner contrary to selection. In Generation 29, the weight of the large stock selected for breeding purposes exceeded the mean of their generation by 6 gm. Yet, in spite of this selection, the average weight for Generation 30 was almost five grams lighter than the mean for Generation 29. In almost every case no member of the litter was as large as its parents. Selection for small body size also appears to have reached its limit. In this case the smaller individuals selected for parent stock would not breed, so succeeding generations had to be bred from average rather than extreme individuals.

The large strain (L), used as a parental stock, is the result of 30 generations of selection for greater body size. Beginning with a stock whose males averaged 23 gm., MacArthur (4) had males in the 21st generation which averaged 39.8 gm. Progress after this generation was slow and erratic, so that while a

maximum weight of 42 gm. was reached in Generation 28, it was impossible to maintain this high level. The variance show great heterogeneity, ranging from a low of 5.84 to a high of 30.87 for groups of comparable size. Generation 32 showed the least variability, and it was from this stock that parents for cross No. 3 were taken. In general the variances for later generations are smaller than those for earlier ones, this is probably because the variance increases and decreases with body size when the present scale is used.

In the L strain the average litter size at birth varies in the different generation from 9.0 to 11.3. The survival at 60 days ranges from 6.9 to 9.1 so that approximately 80% of the young born are utilized for body size data.

In the small strain (S) there are fewer generations and fewer mice per generation. This is partly because of litter size, and partly because this strain does not breed as readily as the L mice. The variance in this strain is much less than in the L strain, and would indicate that environmental variability as measured on this scale varies with body weight. The number of young at birth was very uniform, ranging from 5.1 to 5.8 young at birth with a survival rate similar to that of the L strain.

The *DBa*^{t} parents were the mass produced descendants of the inbred *C57Bl-a*^{t} line, while the *pbs* parents were a new strain which had been inbred for five generations. The weights in Table I indicate that while there is a great deal of variation between the mean weights for subsequent generations of the same strain, yet the size differential between strains is such that we have three parental types; large, small, and intermediate.

In selecting the parent strains for this experiment it was realized that low genetic variability was a highly desirable characteristic. At first glance, inbred strains should be the answer to this. However, it must be remembered that inbreeding without selection will not cause loss in genetic variability. The effect of consanguineous mating is merely to form a heterogeneous population of inbred lines. Many of these lines may be discarded because of lack of space or fertility, and thus achieve the result of direct selection. Most inbred stocks have never been selected for body size and may therefore consist of somewhat heterogeneous material as far as size polygenes are concerned. Furthermore, one must recognize the fact that a reduction in body size usually accompanies inbreeding, so that the inbreeding depression may complicate experiments instead of simplifying them.

The amount of variability present in an inbred line was investigated by compiling data for *C57BL/T* which has been inbred by brother × sister mating for 32 generations. The males weighed 18.8 gm. and the females 15.9 gm. The variance for 49 males and 37 females was 6.7 and 2.8 respectively. This is as large as the noninbred *S* strain. When females from the F_2 of L × S are crossed with *C57BL* in order to introduce a uniform background for comparison, the body size of the progeny is much larger than expected. This could indicate that the phenotypic size of the *C57BL* is not a good indicator of their genotype.

As a further check of the effects of inbreeding in the earlier generations, two sets of data for *pbs* are given in Table I. The first row of values was taken at the end of the fifth generation of brother × sister mating, at which time they were used as parent material for two crosses. The next line gives the same data for the sixth to ninth generation. It will be seen that four more generations of inbreeding produced no change in either the mean or variance of the males, but in the females body size increased significantly and the variance decreased to less than half its former value. This sexual differential in response is evident in a number of weights given in this paper.

The Criterion of Body Size

In conducting experiments in size inheritance some thought must be given to the measurements taken as the criterion of size. In the case of mice there are two main choices, we can use either weights or lengths. Body length, tail length, and length of the long bones are often used in taxonomic studies but such measurements are better adapted to use with dead mice than with live ones. In order to get complete relaxation for taking body measurements it is necessary to etherize. This is a risky procedure with breeding stock. Length has an advantage over weight in that it is not as much affected by obesity and other metabolic disturbances, but because of the practical difficulties in taking measurements it was decided to use weight, as the exclusive criterion of body size.

Having decided to use weights, the next point to be settled is when to take these weights. In his selection experiments, MacArthur (4), took weights at 30 and 60 days and used these as the criteria of size. Butler and Metrakos (1) weighed a fairly extensive series of the parent strains used in this experiment and give the growth curves up to 140 days. At certain selected points they also give the standard errors of the lines. From their Fig. 11 it will be seen that the smallest standard error or the most reliable measurements occur at about 60 days. In the first 20 days the growth rate depends upon the milk supply of the female, while in the 20 to 30 day period, growth depends upon how well the mice adjust themselves to the changeover from milk to a solid diet. Deficiencies and excesses of one period are often ironed out in the following periods. "Peck order" may often play an important part in early growth. By 60 days, growth is fairly well completed and stabilized. After 60 days obesity often enters the picture and some mice tend to put on or lose fat at various stages of their reproductive cycles.

As an example of the results which can be obtained by weighing mice at different ages, the weights of six males from an F_1 litter of the cross L × S are plotted in Fig. 1. It will be seen from this figure that the growth curves are not parallel but cross one another, thus changing the rank order for size. In the 20-day weights the heaviest mouse is No. 2 with No. 1 in fourth place. Later No. 1 becomes the heaviest mouse reaching a maximum weight at 80 days and then losing a few grams before 90 days. This regression in weight

is characteristic of certain mice and is usually only 2 or 3 gm. Sometimes all the males in a litter will show similar decreases in weight at the same time although there is no corresponding change in the environment.

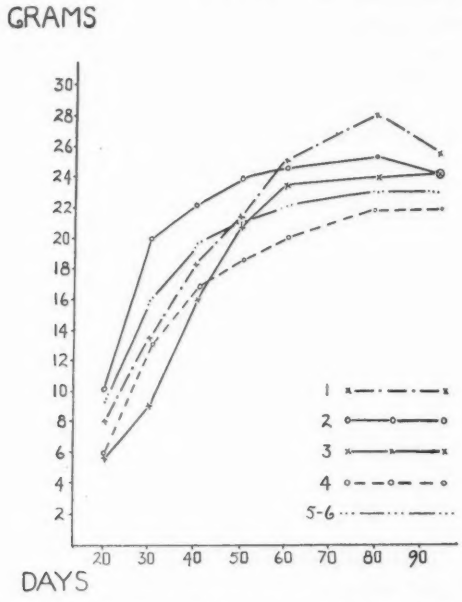

FIG. 1. Growth curves for six male littermates. Data from the F_1 of L × S.

A study of the growth data for the various crosses indicates that there are differences in growth rate between the 40th and 60th day. Whether such differences are environmental, or inherited, has not been determined. By taking 60-day weights as the criterion of body size we are probably only measuring ability to reach a certain size by 60 days. We do not differentiate between ones which grow at a fast rate to 40 days and then at a slow rate to maturity; slow to 40 and then fast; or at a moderate rate all through.

Sexual Differences in Body Weight

In size inheritance studies with bisexual organisms the use of one weight for males and another for females is clumsy. Furthermore a partitioning of the effect of litter size, or of litter weight differences between parents is complicated by variations in the sex ratio. For this reason it is better to have one standard weight to represent both sexes. It has been customary to use male-equivalents and bring the female measurements to the same scale by adding a constant, or multiplying by a conversion figure. Such a measurement works fairly well when the difference between the sexes is constant over the whole range of the experiment. If the difference is not constant, the mid-sex figure

gives a better representation of the data. The mid-sex weight is the weight midway between the mean weights for each sex. The use of a standard measurement eliminates from the total sum of the squares the between-sexes portion and allows comparisons to be made without consideration of the sex ratio.

The average weight of the males is greater than the average weight of the females of the same strain. The amount by which the males exceed the females is not the same for all strains. In general, it varies directly with body weight. In Fig. 2 are plotted the average measurements for males on the abscissa, and

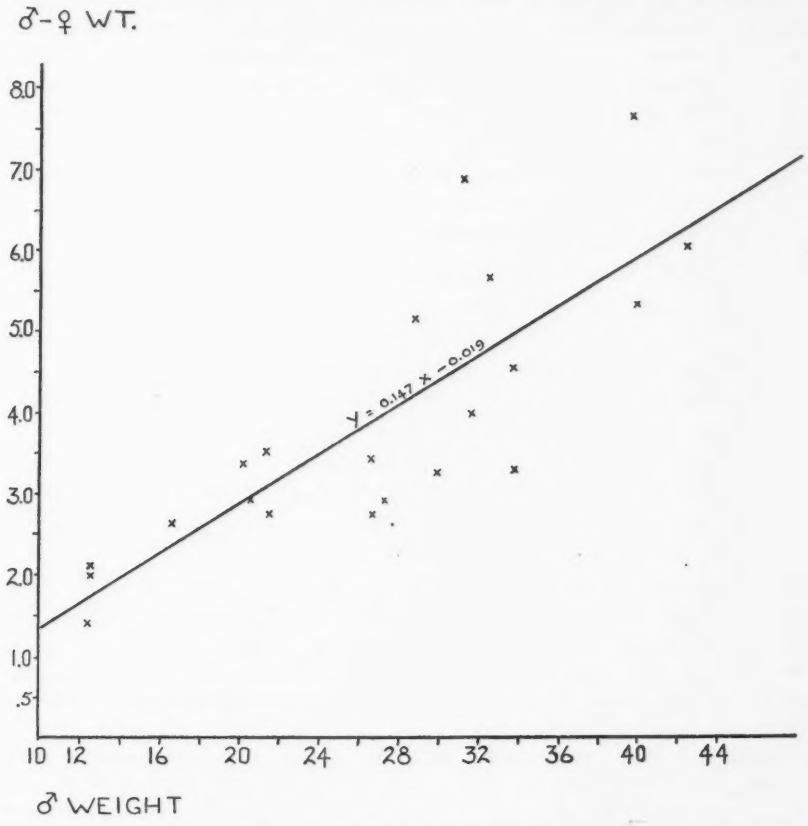

FIG. 2. The relationship between the body weights of males and females of the same strain.

male minus female weight on the ordinate for all generations used in these experiments. The line of best fit is $Y=0.147X-0.019$. Thus a 10 gm. male would average 1.45 gm. heavier than females of the same strain while this difference would increase to 2.92 for a 20 gm. male, 4.39 for a 30 gm., and 5.86 for a 40 gm. male.

Whether or not the relationship between ♂ and ♀ weights given by the above equation is characteristic of *Mus musculus* cannot be proved from these data. There is some evidence that the offspring of certain matings differ from this pattern but not enough data were collected to be sure that such values were not chance ones. More data are needed on this point because the proportionate nature of these differences may give an important clue to the inheritance of body size. Why do these differences exist? Do the genes associated with maleness cause the existing size genes to produce a greater effect than they do in the presence of the female-producing genes? Or is it a pleiotropic effect, or the result of linkage of size genes with sex genes? The answer to these questions must await further experiments of more refined design, but irrespective of the mechanism the important fact stands out that the sex difference in 60 day weight is proportionate when compared on a gram scale. The male-determining genes or ones associated with these do not add a definite number of grams to body size but add about 15% to body weight. This proportionate instead of additive effect is of importance and will be considered again when we take up scaling. There is a suggestion that in the F_2 there is less difference between the sexes than in the F_1 of similar size.

The Effect of Litter Sequence on Body Size

The average litter size changes with successive parturitions; usually the second litter is the largest (Grüneberg (3)). In size inheritance studies the single litter often contains too few individuals to give sufficient data on a generation, or to provide adequate breeding stock. Consequently it is desirable to ascertain whether successive litters from the same parents can be grouped together without significantly increasing the environmental variance.

To test this point we have available two groups of L strain. The five females of one group each have three litters from identical matings. The second group of eight females have four litters each from the same mating. Neither group shows the expected differences in litter size, the successive litters in the one group being 12, 10, and 11, while in the other group they were 11, 10, 12, and 12. These 47 litters show no significant differences in litter size or survival rate.

The weights were analyzed for their various components, with the following results:—

Source	Degrees of freedom	Mean square	*F*
Between litters	14	61.32	6.44**
Between parental groups	4	64.59	6.78**
Within parental groups	10	60.02	6.30**
Between parturitions	2	23.31	2.44
Within litters	111	9.52	

This analysis shows that a significant amount of the variance is between litters and that this can be further subdivided into that contributed by the five parental groups and that within a group of three successive litters by the same parents. These sources contribute approximately the same amounts to the total variance. The differences between successive parturitions is small and not significant.

In the group with four litters per parent the mean square between parturitions is 2.36 for three degrees of freedom which is much smaller than the within-parturition groups' mean square of 88.82 for 28 degrees of freedom. So these data also indicate that it is possible to combine litters from successive matings of the same parents and treat them as one litter. The foregoing should not be taken to mean that there are no differences between successive litters with the same parents, because occasionally large differences do occur. Such differences are probably connected with the health of the female since they occur more often when mice are weighed at 30 days.

The Effect of Litter Size on Weight

The number of young per litter varies greatly even within the same strain. The problem arises, does the size of litter have any effect on body weight? It might be supposed that larger litters would be more poorly nourished in both the uterine and the nursing periods (Falconer (2)). Furthermore, since we are dealing with weights at 60 days, we expect that some of the small litters are the result of mortality within that litter. Such small litters would either be handicapped if mortality was due to lack of milk, or favored if mortality was the result of some other cause. The investigation of the effect of the number of sibs on body size is complicated by the fact that litter size is also an inherited character. Effects ascribed to litter size may really be due to the differences in dams.

The effect of litter size was investigated in both P_1 and F_1 data. In the F_1 of the cross L × *pbs* there were 19 litters which varied in size from 6 to 15 young. The results of this analysis were:—

Source	S.S.	D.F.	M.S.	*F*
Between litters	1969.7	18	109.42	
Between sizes of litters	526.79	8	65.85	2.19
Between litters of the same size	1442.91	10	144.29	34.68**
Within litters	682.89	164	4.16	

It will be seen that the mean square for litter size is smaller than the mean square between litters of the same size, so that litter size has no significant effect on body size. These results are typical of seven other analyses and we can conclude that litter size is of minor importance and can profitably be neglected.

Adequacy of the Scale Used in Determining Inheritance

Having decided to use gram weights at 60 days as the criterion of body size we must next determine whether the measurements can be used in any or all crosses as grams or whether they must be transformed to some other scale. MacArthur and Butler (5) showed that if one measurement fitted an additive scheme, then squares, roots, and cubes of this number certainly could not fit the same scheme. Thus it is important at the outset to choose a scale which will simplify genetic analysis. Mather (6) has shown that scaling is extremely important and that the original measurements and not the derived statistics must be changed to the scale adopted. On the ideal scale, genic effects must on the average be simply additive; and the contribution made to body size by the nonheritable agents must be independent of the genotype. Thus the average effect of gene A or polygene group A will be the same irrespective of whether it is in the genotype with B or b, or whether it is raised in a good or bad environment.

If for simplicity we treat the parents as pure-breeding strains and utilize the symbols and statistics of Mather (6, page 44), we get the results tabulated in Table II. The weights used are the mid-sex weights and the errors are found by pooling the two sums of squares.

In cross No. 1 the constants B and C both have significant negative values and A is almost significant. The main cause of these departures from zero is the high F_1 value. If we infer that heterosis inflates the F_1 value by two grams, then $A=-0.66$, $B=-0.1$, and $C=-3.1$ with only the last value significant. Cross No. 2 has a significant negative A value but the backcross to the large parent was not made, so no test of B is possible. Cross No. 3 with the largest range shows significant differences between reciprocals in both the F_1 and F_2. Because of this it is necessary to calculate two sets of constants. The backcrosses consist of two litters only, so no errors have been calculated. Most of the constants differ significantly from the expected value of zero. In crosses No. 4 and No. 5 the constants are large but not significant.

The results in Table II indicate that as far as these criteria are concerned the scale is not adequate except possibly for crosses No. 4 and No. 5. Examination of the variances of the parents and F_2's of the different crosses shows no difference in magnitude of this statistic for the two generations, which is a point in favor of this scale.

There are three lines of evidence which indicate that a simple gram scale is not adequate. First, the difference in weight between males and females in any strain is proportionate to the average weight of the strain. The second line of evidence comes from MacArthur's selection experiments. Beginning with a mid-sex weight of 21.33 gm., and selecting for both large and small body size, he found that after 21 generations the large selection was 37.15 and the small one 11.38. Thus apparently equal selection pressure resulted in an increase of 15.83 gm., and a decrease of 9.95 gm. If for simplicity we postulate the selection of equal numbers of plus genes in the large strain and minus genes in the small strain then it appears that plus genes are either more

TABLE II

TESTS FOR THE ADEQUACY OF THE SCALE USING MID-SEX WEIGHTS

	Cross No. 1, L × *DBa*^{t}	Cross No. 2, L × *pbs*	Cross No. 3, L × S	Cross No. 4, *DBa*^{t} × S	Cross No. 5, *pbs* × S
Weights in grams					
$\bar{P}_1$	20.6±0.25	20.0±0.24	11.9±0.15	13.0±0.36	11.9±0.15
$\bar{B}_1$	25.5±0.28	21.3±0.27	17.2	15.3±0.42	
$\bar{F}_2$	25.7±0.22	24 8±0.12	19.5±0.24 22.7±0.24	18.4±0.61	19.0±0.19
$\bar{F}_1$	29.0±0.29	25.4±0.27	18.2±0.38 20.8±0.31	18.3±0.50	19.4±1.20
$\bar{B}_2$	29.1±0.22		26.0	19.5±0.50	
$\bar{P}_2$	31.3±0.68	29.4±0.30	32.0±0.20	20.6±0.25	20.0±0.24
A	1.34±0.68	−3.0±0.65	4.3± 1.7	−0.79±1.04	
B	−2.10±1.01		1.8 −0.8	0.04±1.04	
C	−7.10±1.38	−1.2±0.82	−2.3±1.2 5.3±1.3	3.39±2.67	5.3±2.9

	Cross No. 1	Cross No. 2	Cross No. 3
Weights in log-grams			
$\bar{P}_1$	1.301±0.0065	1.295±0.0068	1.070±0.0059
$\bar{B}_1$	1.400±0.0061	1.322±0.0056	1.212
$\bar{F}_2$	1.415±0.0036	1.399±0.0022	1.299±0.0047
$\bar{F}_1$	1.459±0.0050	1.400±0.0048	1.312±0.0066
$\bar{B}_2$	1.456±0.0048		1.406
$\bar{P}_2$	1.501±0.0039	1.504±0.0036	1.503±0.0041
A	0.040±0.010	−0.051±0.014	0.042
B	−0.048±0.012		−0.003
C	−0.060±0.017	−0.003±0.012	0.001±0.024

$A = 2\bar{B}_1 - \bar{P}_1 - \bar{F}_1$; $B = 2\bar{B}_2 - \bar{P}_2 - \bar{F}_1$; $C = 4\bar{F}_2 - 2\bar{F}_1 - \bar{P}_1 - \bar{P}_2$.

effective than minus ones or else are proportionate instead of additive in their action. The third line of evidence is from an inspection of the variances. These variances increase with body size so that either the environmental

portion, or the heredity portion, or both, have effects which are not independent of body size. In this paper we have made no attempt to separate the environmental and hereditary effects, but since the S strain has a variance which is smaller than the variance of the larger inbreds we know that the environmental variance in this case must vary with body size.

Since the gram scale is not adequate, we have transformed the measurements to logarithms, and recalculated the statistics for the first three crosses. The procedure used was not strictly correct since we used the logarithms of the mid-sex weights and these had been calculated arithmetically, but in this preliminary analysis the error involved should not be great enough to invalidate the conclusions. In crosses No. 1 and No. 2 the constants calculated from the logarithms of the mid-sex weights give no better fit than did those calculated from the gram weights. In cross No. 3 the logarithmic data give a much better fit but in the absence of sizable backcrosses it is not possible to test *A* and *B* for significance. In calculating the constants for cross No. 3 the F_1 and F_2 data were lumped instead of doing each reciprocal cross separately and this accounts partly for the good fit of *C*. The variances calculated from the logs are inversely proportional to body size which would indicate that the use of logs overcorrects the proportional effects seen in the gram weights. It would appear from these limited data that the use of a multiplicative constant such as .85 (representing the proportionality between the sexes) might give a better fit. In the meantime, because of the labor involved, these crosses will be reported in the original gram weights.

Cross No. 1, L × *DBa*^{t}

This cross originated from the mating of a large strain male with three sisters from a black and tan (*DBa*^{t}) inbred strain. From the hybrid generation four males were taken and mated with their sisters. In one case the sisters were not litter-mates. A breakdown of the sources of variability gives the following results:—

Source	S.S.	D.F.	M.S.	*F*
Total	1864.8	185		
Between sires	385.5	3	128.5	20.4**
Between litters with same sire	415.1	13	31.9	5.1**
Within litters	1064.2	169	6.3	

It will be seen from this that there are significant differences between the offspring of the four sires and also between the litters with the same sire. Unfortunately the mating system was kept rigid and diallele crosses were not made, so the variability could not be further analyzed. It was noted that the two heaviest sires produced males with the smallest average weight.

The most conspicuous feature about this cross was the rapid growth of the hybrid young. When they were 30 days old they were as large as the large

strain mice of the same age. From 30 to 60 days the growth of the hybrids was much slower than in the large strain so that at 60 days there was a difference of 2 to 3 gm.

In this cross there was segregation for black and brown, intense and dilute, agouti and non-agouti, and of albino and colored. Since MacArthur's (4) selection experiment had resulted in the production of a large strain that was dilute brown agouti or albino and a small strain that was black or black agouti, he suggested that body size was associated with these coat color genes. Accordingly the F_2 could be expected to show differences in body size between the segregate classes. No such differences could be found. In fact the slight differences observed were in the opposite direction to that expected; the black were slightly heavier than brown, and the intense were heavier than dilute. None of the differences were significant.

TABLE III

WEIGHT AND LITTER DATA FOR CROSS NO. 1 (L × *DBa*^{t}) AND CROSS NO. 4 (S × *DBa*^{t})

	Males		Females		Litter size	
	No.	Mean	No.	Mean	Birth	60 days
Parents						
Large strain	79	33.6±0.45	77	29.1±0.32	10.2	8.8
Small strain	27	13.7±0.57	19	12.3±0.36	5.1	4.0
DBa^t	95	22.4±0.34	86	18.8±0.37	6.8	6.0
Cross No. 1. Large × *DBa*^{t}						
F_1 (L male)	40	31.8±0.44	33	26.2±0.36	7.5	7.0
F_2	121	27.9±0.35	114	23.6±0.28	13.2	11.6
B_1	16	26.7±0.42	15	24.3±0.38		
B_2	88	31.4±0.31	75	26.2±0.32	13.1	12.1
Cross No. 4. Small × *DBa*^{t}						
F_1 (S male)	6	20.0±0.78	6	16.7±0.78	6.0	6.0
F_2	57	20.1±0.31	75	16.8±0.24	7.9	7.2
B_1	24	16.6±0.42	11	14.0±0.59	7.5	6.9
B_2	8	20.5±0.79	6	18.5±0.75		

The litter sizes in this cross are worthy of comment. The parents had average litters of 10.2 and 6.8 while the F_1 were derived from litters of 7.5 young. Thus the hybrid young came from litters similar in size to the pure *DBa*^{t} female strain, the male having little or no influence on litter size. Litters produced by the F_1 females had an average size of 13.2 young or 30% better than the large parent. In the backcross (B_2) the litter size was equally large no matter whether the F_1 or the L strain was used for the female parent. Corpora lutea counts of the L strain gave an average of 15.2 so the following interpretation is offered. In the L strain, of 15 eggs shed, only 10 reach full term, but if an F_1 male is used the extra vigor leads to a greater survival and more young are born. There were no reciprocal crosses made for the F_1 or

they might have shed more light on this point. The simplest explanation of litter size inheritance in this cross would be that the large strain potential of 15 young is inherited as a dominant. Segregation would not show up unless complex crosses were made involving the F_2 mice. The data for this cross are given in Table III.

Cross No. 4, *DBa*^{t} × Small Strain

In cross No. 4 which was made in one direction only, a small strain male was mated to two *DBa*^{t} females. The F_1 and F_2 were almost identical in weights, the males averaging 2 gm. heavier than the mid-point between the S and *DBa*^{t} males, and the females 1 gm. heavier than the mid-point between the S and *DBa*^{t} females. The B_1 backcross was exactly intermediate between the F_1 and the small parent. The litter sizes are again slightly larger than in the parent strains but in this case there is not much difference between the litter sizes of the two parents. The data are given in Table III.

Cross No. 2, Large Strain × *pbs*

The F_1 of this cross was produced by mating four *pbs* males to 20 large strain females. Of the males, M108 and M167, came from four generations of brother × sister mating, M170 from five generations and M213 from six generations of inbreeding. Twenty-three litters were produced with an average weight of 26.7 for males and 24.1 for females. The outstanding point is the large variance in the F_1 and the significant difference between the offspring sired by each male as seen in the following analysis:—

Source	S.S.	D.F.	M.S.	*F*
Total	2652.59	182	14.57	
Between sires	1371.46	3	457.15	11.4**
Between litters with the same sire	598.24	15	39.88	9.9**
Within litters	682.89	164	4.16	

From this we see that more than half of the F_1 variance is the result of the use of more than one sire. Since the females were assigned at random to each male, the greatest part of this difference is due to the genetic constitution of the male. Because of these significant differences between sires it seems advisable to split both the F_1 and F_2 up into similar groups (Table IV). M108, the male with the least inbreeding, produced the heaviest F_1 progeny. The variance is similar to what would be expected in parent strains of the same size.

In the total F_2 generation the mean of the males is similar to that of the F_1, while that of the females is 1 gm. lighter than the F_1. Again we find significant differences between the means of the various sire groups. In this case the grouping has real significance since each male is mated to his own sisters.

The following analysis is typical of these groups:—

	S.S.	D.F.	M.S.
Between dams	137.21	6	22.86
Between litters with same dam	288.77	11	26.25
Within litters	557.22	119	4.68

TABLE IV

BODY WEIGHTS AND VARIANCES FOR CROSS NO. 2 (LARGE × *pbs*)

Male No.	Males			Females		
	Number	Mean	Variance	Number	Mean	Variance
F_1 generation						
M108	11	33.0	11.7	8	29.2	11.6
M167	20	28.4	4.50	24	26.5	7.43
M170	39	24.4	10.0	38	21.2	3.70
M213	26	26.2	7.46	17	24.7	7.05
Total	96	26.7	15.[illegible]	87	24.1	13.5
F_2 generation						
M171 (ex M108)	64	25.8	12.2	46	22.3	9.51
M195 (ex M108)	45	28.4	15.5	34	25.1	16.9
M220 (ex M167)	22	26.5	8.31	21	23.3	7.75
M221 (ex M167)	22	24.1	6.47	19	20.9	7.96
M226 (ex M167)	61	26.3	4.80	76	22.4	4.61
M237 (ex M213)	31	26.6	9.72	17	23.3	7.93
M248 (ex M170)	48	26.3	10.1	45	23.4	8.01
M255 (ex M170)	25	26.8	14.2	19	24.9	8.43
Total	318	26.5	10.4	277	23.1	9.76
B_1 (F_1 × *pbs*)						
Total	73	21.6	11.4	66	20.9	8.85

The main point brought out by these analyses is that the within-litter component is similar in all cases. This component is also of the same magnitude as the within of the F_1, but smaller than the P_1 variance of 8.49 for the L strain. In the M171 and M195 groups, the variance between dams is larger than the mean square between litters of the same parentage. It is interesting to note that both of these groups came from the F_1 sired by M108 which showed the largest variance in the F_1. Each of the three sources,—between sires, between dams, and between litters of the same parentage, contribute significant amounts to the total variability.

The results of this cross are shown graphically in Fig. 3. The large parent mean is 50% heavier than the mean of the smaller parent, but the variability in both stocks is such that there is a definite overlap in the 21-26 gm. range. The range in the F_1, F_2, and B_1 is approximately the same instead of the F_2 range being the largest and the F_1 range the smallest.

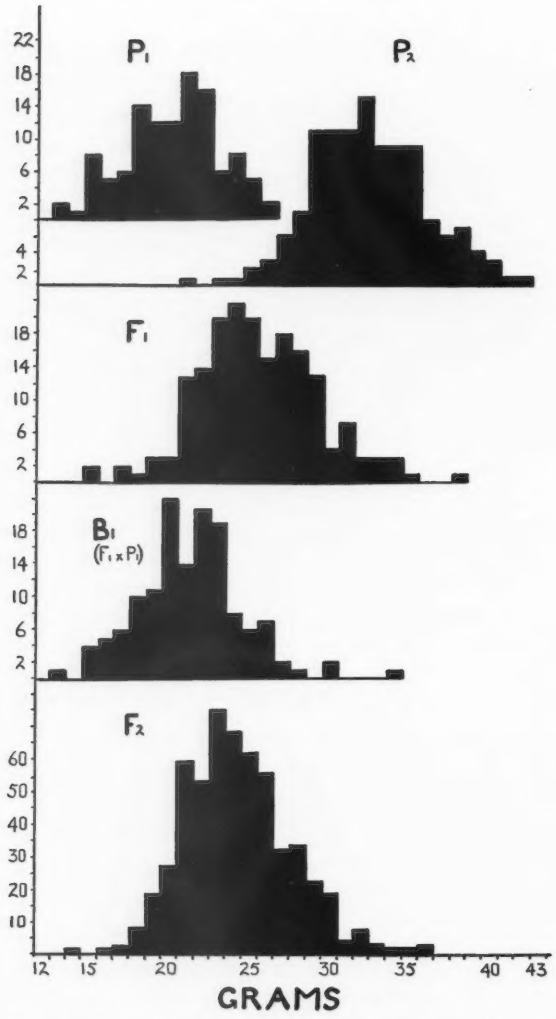

FIG. 3. The frequency distributions for different generations of the cross L × *pbs*.

Cross No. 3, Large Strain × Small Strain

In cross No. 3 the largest of the parent strains was crossed with the smallest of these strains, so this cross is most liable to clearly show the effects of heredity. The results of this cross are given in Table V and the frequency distribution is illustrated in Fig. 4. It is noticed immediately that there are dissimilarities between reciprocal crosses. In the F_1 the difference between reciprocal crosses is 3.1 ± 1.0 for males and 2.0 ± 0.58 for

TABLE V

WEIGHTS AND LITTER SIZES FOR CROSSES No. 3 (L × S) AND No. 5 (S × *pbs*)

	Males		Females		Litter size	
	Number	Mean	Number	Mean	Birth	60 days
Parents						
Large strain	108	33.7±0.31	76	30.3±0.28	8.0	7.4
Small strain	48	13.1±0.21	60	10.8±0.21	5.5	4.3
F_1 generation						
L × S female	5	19.1±0.91	8	17.4±0.32	4.6	4.3
L × S male	51	22.2±0.41	33	19.4±0.48	11.2	8.4
F_2 generation						
L × S female F_1	67	21.4±0.39	65	17.8±0.30	8.5	7.1
L × S male F_1	45	23.6±0.41	45	21.7±0.48	7.3	6.0
B_1 (F_1 × S female)	3	17.7	2	16.7		
B_2 (F_1 × L female)	5	26.6	5	25.4		
McGill F_1						
L × S female	7	23.1±1.0	3	18.6±1.2		
MacArthur's cross						
L × S male F_1	11	26.4±0.99	11	17.6±0.63		
L × S male F_2	23	20.9±0.56	32	20.6±0.60		
S × *pbs*						
F_1	5	19.9±1.3	8	18.9±1.9		
F_2	105	20.5±0.24	101	17.6±0.29		

females, both highly significant. Because of the apparent heterogeneity of the parent strains we cannot say with certainty that this difference is environmental, but it would appear that the larger body of the L strain female furnishes a better uterine environment the effect of which is not lost in the postnatal period. This environmental effect accounts for a 2 to 3 gm. or 15-20% increase at 60 days. The possibility of this difference being due to the superiority of the L strain milk supply has been ruled out in a previous paper (Butler and Metrakos (1)). It was shown at that time that milk from the L strain was not superior to milk from S and that most weight differences due to milk supply and quality were transitory. Unfortunately the cross of L × S female was confined to three litters but the results are suggestive enough that adequately controlled reciprocal crosses will be included in future experiments. From observation of the litter sizes one would expect that the F_1 offspring of the S strain female would be favored, since the S females produced average litters of only 4.6 hybrid young compared with the 11.2 hybrid young produced by L females.

In the F_2 there is still a dissimilarity between reciprocal crosses, the F_1 from the L female giving larger F_2 offspring. In the males the difference was 2.2±0.61 and in the females 3.9±0.56. The difference in males could

possibly be explained as environmental, resulting from the growth of the embryos in a larger uterus. The difference in females on the other hand is too great for such an explanation. It will be observed that the weight differences between the two sexes vary greatly in these reciprocals. In the F_2 from

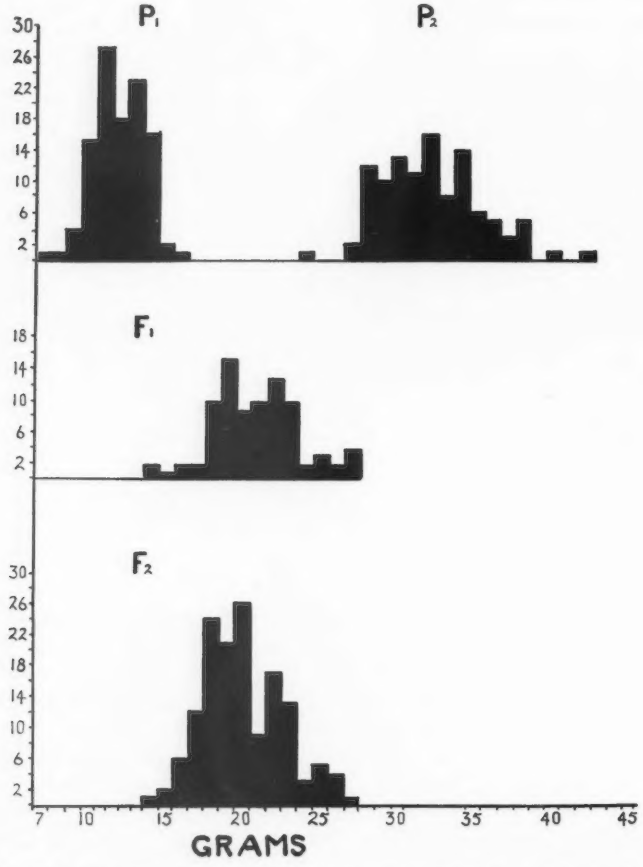

FIG. 4. The frequency distributions for different generations of the cross L × S.

the S female the males are 3.6 gm. larger than the females, while in the reciprocal cross the males are only 1.9 gm. larger than the females. Thus in the one case the difference is greater than expected and in the other case less than expected on the basis of the equation derived earlier in this paper. These observations suggest that there was a genetic difference between reciprocals and possibly a sex-associated body size segregation. This point is worth pursuing when adequate data become available. The data from a less

numerous cross made at McGill, and one made by MacArthur, both using a large strain of greater body size, give mean weights of similar magnitude.

The litter size data is not as worthy of comment as those of cross No. 2 but here again it appears that the production of hybrid young increases the litter size produced by the L female. On the other hand the S strain female produced the same size of litter irrespective of whether the young are pure S or hybrid LS.

Cross No. 5, S × *pbs*

The most significant point about this cross is that the F_1 and F_2 are both close to the mean weight of the *pbs* or larger parent. Rescaling is certainly necessary in this case but in the absence of backcross data it has not been attempted.

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